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Invited Review

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Monitoring and Safety of Azathioprine Therapy in Inflammatory Bowel Disease

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Azathioprine is the most common drug used to maintain clinical remission in inflammatory bowel disease. This drug is also important as a steroid-sparing agent in steroid-dependent and chronically active inflammatory bowel disease. Nevertheless, many questions remain concerning the optimal treatment regimens of azathioprine. The dose of azathioprine has to be reduced or the therapy has to be discontinued frequently because of drug-induced toxicity. In this review, we discuss monitoring of thiopurines, adverse events, malignant complications and how to use azathioprine safely and usefully. **(Pediatr Gastroenterol Hepatol Nutr 2013; 16: 65～70)**

Key Words: Azathioprine, Inflammatory bowel diseases

INTRODUCTION

Thiopurines used for inflammatory bowel disease (IBD) treatment are azathioprine (AZA) and 6-mercaptopurine (6-MP). AZA is the most common drug used to maintain clinical remission in Crohn's disease and ulcerative colitis [1,2]. This drug is also important as a steroid-sparing agent in steroid-dependent and chronically active IBD. However, the dose of AZA has to be reduced or the therapy has to be discontinued in 9-28% of patients because of drug-induced toxicity [3]. Bone marrow suppression, gastrointestinal disturbances, hepatotoxicity, pancreatitis, fever and rash are among the most frequent reasons for AZA reduction/cessation in some patients [4]. Although there are some adverse reactions that clinicians should be aware of, monitoring 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine (6-MMP) levels enables safe and long-term remission in IBD patients [5,6].

METABOLISM AND MONITORING OF THIOPURINES

Thiopurines undergo complex metabolism. Three important enzymes of xanthine oxidase (XO), thiopurine S-methyl transferase (TPMT), and hypoxanthine phosphoribosyl transferase (HPRT) act

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competitively in the process of converting AZA/6-MP to inactive and active metabolites [7,8]. AZA is converted via an non-enzymatic reaction to 6-MP, which is subsequently metabolized either through TPMT to 6-MMP or, alternatively, by XO to thiouric acid. Furthermore, 6-MP may be converted by HPRT to thioinosine monophosphate. Subsequently, the enzyme inosine triphosphate pyrophosphatase (ITPA) catalyses the trivial cycle to thioinosine triphosphate and conversely, to avoid the accumulation of thioinosine monophosphate, and from guanosine monophosphate synthetase to 6-TGN [9-12].

6-TGN appears to be one of the active metabolites responsible for therapeutic efficacy. Several studies have found significant correlations between 6-TGN concentration and clinical response in IBD and use a therapeutic range for 6-TGN concentration of 235-450 pmol/8×10^8 red blood cells (RBC) [6,13-15]. A 6-TGN concentration of >235 pmol/8×10^8 RBC is associated with clinical response [6,13]. The cutoff concentration above 450 pmol/8×10^8 RBC is based on an increased risk of side effects (myelotoxicity and nodular regenerative hyperplasia of the liver) without an increase in efficacy [15,16]. Patients with erythrocyte 6-MMP concentrations above 5,700 pmol/8×10^8 RBC are at increased risk of hepatotoxicity and are unlikely to respond to treatment by increasing the drug dose [13,17,18]. These patients are probably preferentially metabolizing AZA via TPMT to form 6-MMP and may benefit from reduction of thiopurine dose by 50-75%, and careful monitoring of hematological indices and metabolites [19].

Dose recommendations for AZA vary slightly between Western guidelines, with a daily dose of 2-3 mg/kg AZA recommended by the American Gastroenterological Association (AGA) [20], and a daily dose of 1.5-2.5 mg/kg AZA recommended by the European Crohn's and Colitis Organisation (ECCO) [21]. However, these recommendations do not necessarily hold true for other ethnicities. Several Japanese studies showed that Japanese IBD patients might reach sufficient 6-TGN values with substantially lower AZA dosages in adults, children and adolescents [22-24].

A number of coadministered drugs may potentially influence thiopurine metabolism [7,10,25]. In vitro studies have confirmed that 5-aminosalicylic acid compounds are inhibitors of TPMT [26,27]. The higher frequency of leucopenia is observed in patients using this combination [26]. Other frequently prescribed TPMT inhibitors include acetylsalicylic acid and furosemide [7]. Allopurinol inhibits XO, resulting in increase in 6-TGN concentrations [28]. Roblin et al. [29] reported interactions between AZA and infliximab. They observed that the mean 6-TGN level was significantly increased within 1-3 weeks after the first infliximab infusion, and a decrement in leucocyte count. These modifications were normalized 3 months after infusion.

TPMT MONITORING

TPMT is the most frequently studied enzyme of AZA metabolism and the only one usually tested for in routine clinic. TPMT status can be checked for based on phenotype or genotype tests. TPMT genotyping consists of detecting single nucleotide polymorphism responsible for TPMT inactivation. A good correlation exists between TPMT activity and genotyping [30]. Based on TPMT and genetic polymorphism, the general population can be divided in three groups: wild type homozygous TPMT with high methylation activity (88%), heterozygous for a deficient TPMT allele with intermediate activity (11%) and homozygous for deficient TPMT alleles with a low activity (0.3%) [31]. The human TPMT gene is located on chromosome 6p22.3 and consists of 10 exons and nine introns. To date, 27 alleles responsible for possible TPMT activity deficiency have been described: *2, *3A, *3B, *3C, *3D, and *4 to *25 [32].

It is known that the variation of TPMT mutations in Caucasians is different from that in other ethnic groups [33,34]. TPMT *3A is the most prevalent-mutant allele in Caucasians [35]. On the other hand, TPMT *3C is the most prevalent mutant allele in Japanese and Chinese patients [36-38]. Also in studies about TPMT polymorphisms in the Korean adult population, TPMT *3C is observed as hetero-

zygotic allele and no TPMT *2, *3A, or *3B are observed [39-41].

Patients with low TPMT activity have elevated 6-TGN when treated with standard doses of AZA and are at greatly increased risk of myelosuppression [42,43]. Whereas patients with very high TPMT activity are either resistant to thiopurine drugs due to shunting of AZA down the 6-MMP pathway [35,44,45] or require a high dose to achieve efficacy, but at the risk of hepatotoxicity due to high 6-MMP concentrations [13,17].

A total of 109 patients were evaluated TPMT in Samsung Medical Center (Seoul, Korea). The distribution of the TPMT genotype was as follows: 102 patients had *1/*1 (wild type), one had *3C/*3C (homozygote), four had *1/*3C, one had *1/*6, and one had *1/*16 (heterozygote). The patient with *3C/*3C mutation required low dose of AZA, 0.18 mg/kg/day for maintain an optimal therapeutic range.

ADVERSE EVENTS

Most adverse events occur within the first 3 months [46]. Adverse events of AZA can be divided into dose-dependent, pharmacologically explainable events on one hand and dose-independent, hypersensitivity reactions on the other [7,47]. The first type of adverse events can occur in any time of the treatement, are well-known to be associated with the formation of potentially toxic metabolites. They include myelosuppression, infectious complications, and malignancies [7]. The others often occur within 2-4 weeks after start of treatement and result in symptoms like fever, rash, arthralgia, pancreatitis, hepatitis, and gastrointestinal disturbances [4]. The most common adverse event to reduce the dose of AZA or to discontinue the treatment is myelosuppression [48].

In AZA therapy, one of the potentially serious side effects is bone marrow suppression. AZA-induced myelotoxicity has been attributed to the low activity of TPMT caused by TPMT genetic polymorphism [49,50]. TPMT polymorphism results in greater conversion of AZA to 6-TGN likely due to bone marrow suppression [3,9]. Therefore, the monitoring of 6-TGN concentrations has been reported to be helpful for managing IBD patients undergoing AZA therapy, since it may identify the optimal AZA dose to maximize efficacy while minimizing the risk of toxicity [5,51].

A total of 174 patients with IBD were treated with AZA in Samsung Medical Center from 2002 to 2012. Among them, 98 patients (56.3%) were experienced adverse events of AZA with 136 episodes. Most common adverse event of AZA was bone marrow suppression (27%). Gastrointestinal disturbances (15.5 %) such as anorexia, nausea and vomiting, and hair loss (12.1%) were also frequently observed. Therefore, the dose of AZA was reduced in 31 patients (17.8%) and administration of AZA was stopped in 18 patients (10.3%). These present results show higher adverse event rates than previously reported western studies. The cause of these differences is not clear. One explanation for this might be the ethnic difference. Despite the low-dose (1.25±0.41 mg/kg/day) use than standard dose (2.0-2.5 mg/kg/day), 17.8% of patients were needed to reduce dose of AZA and 10.3% were discontinued AZA treatment.

MALIGNANT COMPLICATIONS

Treatment with AZA is associated with a potential risk of developing lymphoma [52], including hepatosplenic T-cell lymphoma (HSTCL) [53]. AZA might play a role in the development of HSTCL in patients with IBD, and when such treatment is combined with tumor necrosis factor-α inhibitors, the risk might be amplified [54]. The possible role that immunosuppression plays in promoting certain malignancy has been well described. Immunosuppression is associated with HSTCL in approximately 25-30% of reported cases in the general population. Many of these cases have occurred in patients undergoing renal or heart transplantation treated with AZA and prednisone with or without cyclosporine [55-57]. Also, a recent meta-analysis including observational data from 3,891 patients with IBD re-

ported a 4-fold increased risk of lymphoproliferative disease in patients with IBD treated with AZA [52]. Although the relative risk of lymphoma is increased, the absolute risk still remains rather small, and currently available data show that the benefits of AZA used in IBD greatly outweigh its risks [58].

CONCLUSION

AZA has been generally used for treating chronic active lesions or for the maintenance of remission in IBD. The use of AZA in patients with IBD and ways to monitor therapy have been well documented. However, in clinical practice, the possibility of 6-TGN measurement to monitor therapy seems underused. Monitoring 6-TGN concentrations is helpful in developing a therapeutic strategy for IBD patients. Although TPMT genotype and thiopurine metabolite monitoring could not completely explain the thiopurine-induced adverse events, it could be helpful to examine TPMT genotypes before administering AZA and to measure 6-TGN concentrations during prescribing AZA in IBD patients.

REFERENCES

1. Sandborn W, Sutherland L, Pearson D, May G, Modigliani R, Prantera C. Azathioprine or 6-mercaptopurine for inducing remission of Crohn's disease. Cochrane Database Syst Rev 2000;(2):CD000545.
2. Hibi T, Ogata H. Novel pathophysiological concepts of inflammatory bowel disease. J Gastroenterol 2006;41:10-6.
3. Hindorf U, Lindqvist M, Peterson C, Söderkvist P, Ström M, Hjortswang H, et al. Pharmacogenetics during standardised initiation of thiopurine treatment in inflammatory bowel disease. Gut 2006;55:1423-31.
4. Gearry RB, Barclay ML, Burt MJ, Collett JA, Chapman BA. Thiopurine drug adverse effects in a population of New Zealand patients with inflammatory bowel disease. Pharmacoepidemiol Drug Saf 2004;13:563-7.
5. Goldenberg BA, Rawsthorne P, Bernstein CN. The utility of 6-thioguanine metabolite levels in managing patients with inflammatory bowel disease. Am J Gastroenterol 2004;99:1744-8.
6. Cuffari C, Hunt S, Bayless T. Utilisation of erythrocyte 6-thioguanine metabolite levels to optimise azathioprine therapy in patients with inflammatory bowel disease. Gut 2001;48:642-6.
7. Derijks LJ, Gilissen LP, Hooymans PM, Hommes DW. Review article: thiopurines in inflammatory bowel disease. Aliment Pharmacol Ther 2006;24:715-29.
8. Zelinkova Z, Derijks LJ, Stokkers PC, Vogels EW, van Kampen AH, Curvers WL, et al. Inosine triphosphate pyrophosphatase and thiopurine s-methyltransferase genotypes relationship to azathioprine-induced myelosuppression. Clin Gastroenterol Hepatol 2006;4:44-9.
9. Gearry RB, Barclay ML. Azathioprine and 6-mercaptopurine pharmacogenetics and metabolite monitoring in inflammatory bowel disease. J Gastroenterol Hepatol 2005;20:1149-57.
10. Sahasranaman S, Howard D, Roy S. Clinical pharmacology and pharmacogenetics of thiopurines. Eur J Clin Pharmacol 2008;64:753-67.
11. Sandborn WJ. Rational dosing of azathioprine and 6-mercaptopurine. Gut 2001;48:591-2.
12. Palmieri O, Latiano A, Bossa F, Vecchi M, D'Incà R, Guagnozzi D, et al. Sequential evaluation of thiopurine methyltransferase, inosine triphosphate pyrophosphatase, and HPRT1 genes polymorphisms to explain thiopurines' toxicity and efficacy. Aliment Pharmacol Ther 2007;26:737-45.
13. Dubinsky MC, Lamothe S, Yang HY, Targan SR, Sinnett D, Théorêt Y, et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. Gastroenterology 2000;118:705-13.
14. Ooi CY, Bohane TD, Lee D, Naidoo D, Day AS. Thiopurine metabolite monitoring in paediatric inflammatory bowel disease. Aliment Pharmacol Ther 2007;25:941-7.
15. Wright S, Sanders DS, Lobo AJ, Lennard L. Clinical significance of azathioprine active metabolite concentrations in inflammatory bowel disease. Gut 2004;53:1123-8.
16. Dubinsky MC. Optimizing immunomodulator therapy for inflammatory bowel disease. Curr Gastroenterol Rep 2003;5:506-11.
17. Dubinsky MC, Yang H, Hassard PV, Seidman EG, Kam LY, Abreu MT, et al. 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease. Gastroenterology 2002;122:904-15.
18. Derijks LJ, Gilissen LP, Engels LG, Bos LP, Bus PJ, Lohman JJ, et al. Pharmacokinetics of 6-mercaptopurine in patients with inflammatory bowel disease: implications for therapy. Ther Drug Monit 2004;26:311-8.
19. Haines ML, Ajlouni Y, Irving PM, Sparrow MP, Rose R, Gearry RB, et al. Clinical usefulness of therapeutic drug

monitoring of thiopurines in patients with inadequately controlled inflammatory bowel disease. Inflamm Bowel Dis 2011;17:1301-7.
20. Lichtenstein GR, Abreu MT, Cohen R, Tremaine W; American Gastroenterological Association. American Gastroenterological Association Institute medical position statement on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. Gastroenterology 2006;130:935-9.
21. Dignass A, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, et al; European Crohn's and Colitis Organisation (ECCO). The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: current management. J Crohns Colitis 2010;4:28-62.
22. Andoh A, Tsujikawa T, Ban H, Hashimoto T, Bamba S, Ogawa A, et al. Monitoring 6-thioguanine nucleotide concentrations in Japanese patients with inflammatory bowel disease. J Gastroenterol Hepatol 2008;23:1373-7.
23. Komiyama T, Yajima T, Kubota R, Iwao Y, Sakuraba A, Funakoshi S, et al. Lower doses of 6-mercaptopurine/azathioprine bring enough clinical efficacy and therapeutic concentration of erythrocyte 6-mercaptopurine metabolite in Japanese IBD patients. J Crohns Colitis 2008;2:315-21.
24. Ohtsuka Y, Arai K, Aoyagi Y, Fujii T, Yamakawa Y, Ohtani K, et al. Monitoring 6-thioguanine nucleotide concentrations in Japanese children and adolescents with inflammatory bowel disease. J Gastroenterol Hepatol 2010;25:1626-30.
25. Schwab M, Schäffeler E, Marx C, Fischer C, Lang T, Behrens C, et al. Azathioprine therapy and adverse drug reactions in patients with inflammatory bowel disease: impact of thiopurine S-methyltransferase polymorphism. Pharmacogenetics 2002;12:429-36.
26. Lewis LD, Benin A, Szumlanski CL, Otterness DM, Lennard L, Weinshilboum RM, et al. Olsalazine and 6-mercaptopurine-related bone marrow suppression: a possible drug-drug interaction. Clin Pharmacol Ther 1997;62:464-75.
27. Green JR. Balsalazide and azathioprine or 6-mercaptopurine. Gastroenterology 1999;117:1513-4.
28. Kennedy DT, Hayney MS, Lake KD. Azathioprine and allopurinol: the price of an avoidable drug interaction. Ann Pharmacother 1996;30:951-4.
29. Roblin X, Serre-Debeauvais F, Phelip JM, Bessard G, Bonaz B. Drug interaction between infliximab and azathioprine in patients with Crohn's disease. Aliment Pharmacol Ther 2003;18:917-25.
30. Schaeffeler E, Fischer C, Brockmeier D, Wernet D, Moerike K, Eichelbaum M, et al. Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. Pharmacogenetics 2004;14:407-17.
31. Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. Am J Hum Genet 1980;32:651-62.
32. Derijks LJ, Wong DR. Pharmacogenetics of thiopurines in inflammatory bowel disease. Curr Pharm Des 2010;16:145-54.
33. Hon YY, Fessing MY, Pui CH, Relling MV, Krynetski EY, Evans WE. Polymorphism of the thiopurine S-methyltransferase gene in African-Americans. Hum Mol Genet 1999;8:371-6.
34. Otterness D, Szumlanski C, Lennard L, Klemetsdal B, Aarbakke J, Park-Hah JO, et al. Human thiopurine methyltransferase pharmacogenetics: gene sequence polymorphisms. Clin Pharmacol Ther 1997;62:60-73.
35. Yates CR, Krynetski EY, Loennechen T, Fessing MY, Tai HL, Pui CH, et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med 1997;126:608-14.
36. Cao Q, Zhu Q, Shang Y, Gao M, Si J. Thiopurine methyltransferase gene polymorphisms in Chinese patients with inflammatory bowel disease. Digestion 2009;79:58-63.
37. Zhu Q, Cao Q. Thiopurine methyltransferase gene polymorphisms and activity in Chinese patients with inflammatory bowel disease treated with azathioprine. Chin Med J (Engl) 2012;125:3665-70.
38. Hibi T, Naganuma M, Kitahora T, Kinjyo F, Shimoyama T. Low-dose azathioprine is effective and safe for maintenance of remission in patients with ulcerative colitis. J Gastroenterol 2003;38:740-6.
39. Kim S, Lee HW, Lee W, Chun S, Min WK. Validation of new allele-specific real-time PCR system for thiopurine methyltransferase genotyping in Korean population. Biomed Res Int 2013;2013:305704.
40. Kim JH, Cheon JH, Hong SS, Eun CS, Byeon JS, Hong SY, et al. Influences of thiopurine methyltransferase genotype and activity on thiopurine-induced leukopenia in Korean patients with inflammatory bowel disease: a retrospective cohort study. J Clin Gastroenterol 2010;44:e242-8.
41. Jung YS, Cheon JH, Park JJ, Moon CM, Kim ES, Lee JH, et al. Correlation of genotypes for thiopurine methyltransferase and inosine triphosphate pyrophosphatase with long-term clinical outcomes in Korean pa-

tients with inflammatory bowel diseases during treatment with thiopurine drugs. J Hum Genet 2010;55:121-3.
42. Lennard L, Van Loon JA, Lilleyman JS, Weinshilboum RM. Thiopurine pharmacogenetics in leukemia: correlation of erythrocyte thiopurine methyltransferase activity and 6-thioguanine nucleotide concentrations. Clin Pharmacol Ther 1987;41:18-25.
43. Lennard L, Van Loon JA, Weinshilboum RM. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. Clin Pharmacol Ther 1989;46:149-54.
44. Cuffari C, Dassopoulos T, Turnbough L, Thompson RE, Bayless TM. Thiopurine methyltransferase activity influences clinical response to azathioprine in inflammatory bowel disease. Clin Gastroenterol Hepatol 2004;2:410-7.
45. Lennard L, Lilleyman JS, Van Loon J, Weinshilboum RM. Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukaemia. Lancet 1990;336:225-9.
46. Hindorf U, Lindqvist M, Hildebrand H, Fagerberg U, Almer S. Adverse events leading to modification of therapy in a large cohort of patients with inflammatory bowel disease. Aliment Pharmacol Ther 2006;24:331-42.
47. Prefontaine E, Macdonald JK, Sutherland LR. Azathioprine or 6-mercaptopurine for induction of remission in Crohn's disease. Cochrane Database Syst Rev 2009;(4):CD000545.
48. Fraser AG, Orchard TR, Jewell DP. The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. Gut 2002;50:485-9.
49. Derijks LJ, Gilissen LP, Engels LG, Bos LP, Bus PJ, Lohman JJ, et al. Pharmacokinetics of 6-thioguanine in patients with inflammatory bowel disease. Ther Drug Monit 2006;28:45-50.
50. Haglund S, Taipalensuu J, Peterson C, Almer S. IMPDH activity in thiopurine-treated patients with inflammatory bowel disease - relation to TPMT activity and metabolite concentrations. Br J Clin Pharmacol 2008;65:69-77.
51. Gilissen LP, Derijks LJ, Bos LP, Bus PJ, Hooymans PM, Engels LG. Therapeutic drug monitoring in patients with inflammatory bowel disease and established azathioprine therapy. Clin Drug Investig 2004;24:479-86.
52. Kandiel A, Fraser AG, Korelitz BI, Brensinger C, Lewis JD. Increased risk of lymphoma among inflammatory bowel disease patients treated with azathioprine and 6-mercaptopurine. Gut 2005;54:1121-5.
53. Kotlyar DS, Osterman MT, Diamond RH, Porter D, Blonski WC, Wasik M, et al. A systematic review of factors that contribute to hepatosplenic T-cell lymphoma in patients with inflammatory bowel disease. Clin Gastroenterol Hepatol 2011;9:36-41.
54. Shale M, Kanfer E, Panaccione R, Ghosh S. Hepatosplenic T cell lymphoma in inflammatory bowel disease. Gut 2008;57:1639-41.
55. Belhadj K, Reyes F, Farcet JP, Tilly H, Bastard C, Angonin R, et al. Hepatosplenic gammadelta T-cell lymphoma is a rare clinicopathologic entity with poor outcome: report on a series of 21 patients. Blood 2003;102:4261-9.
56. Tey SK, Marlton PV, Hawley CM, Norris D, Gill DS. Post-transplant hepatosplenic T-cell lymphoma successfully treated with HyperCVAD regimen. Am J Hematol 2008;83:330-3.
57. François A, Lesesve JF, Stamatoullas A, Comoz F, Lenormand B, Etienne I, et al. Hepatosplenic gamma/delta T-cell lymphoma: a report of two cases in immunocompromised patients, associated with isochromosome 7q. Am J Surg Pathol 1997;21:781-90.
58. Armstrong RG, West J, Card TR. Risk of cancer in inflammatory bowel disease treated with azathioprine: a UK population-based case-control study. Am J Gastroenterol 2010;105:1604-9.