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ORIGINAL ARTICLE

Xanthine oxidase activity in thiopurine curative Chinese inflammatory bowel disease patients

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Abstract

Xanthine oxidase (XO) competes with thiopurine S-methyltransferase (TPMT) and hypoxanthine guanine phosphoribosyltransferase (HPRT) to metabolize azathioprine (AZA)/6-mercaptopurine (6-MP) in vivo. A retrospective investigation was performed to detect the activity of XO in thiopurine curative Chinese inflammatory bowel disease (IBD) patients. We also evaluated whether a relationship between XO activity and incidence of thiopurine-induced adverse effects (AEs) existed. Clinical data and blood samples were collected from 140 IBD patients before receiving AZA/6-MP therapy, and the erythrocyte XO activity was measured. The XO activities of all patients were 20.29 ± 4.43 U/g Hb. No sex difference in XO activity was observed (p = .728), and the XO activity showed no difference between the UC and CD patients (p = .082). AEs were observed in 41 (29.3%) patients including leukopenia (26, 18.57%), gastrointestinal intolerance (11, 7.86%), flu-like symptom (5, 3.57%), alopecia (5, 3.57%), and hepatotoxicity (1, 0.71%). XO activity was significantly lower in the patients with AEs than in those without AEs (18.40 ± 3.73 vs. 21.07 ± 4.48 U/g Hb, p = .001), especially in the patients with leukopenia (18.29 ± 3.68 vs. 21.07 ± 4.48 U/g Hb, p = .004). However, no significant difference in XO activity was found between patients with and without other AEs. Decreased XO activity was observed in the patients who developed flu-like symptoms (17.58 ± 3.50 U/g Hb) and alopecia (18.67 ± 2.91 U/g Hb) compared

Abbreviations: 5-ASA, 5-aminosalicylic acid; 6-MMP, 6-methylmercaptopurine; 6-MP, 6-mercaptopurine;; 6-TG, 6-thioguanine; 6-TGNs, 6-thioguaninenucleotides; 6-TU, 6-thiouric acid; 8-OHMP, 8-hydroxy-6-mercaptopurine; AEs, adverse effects; AZA, azathioprine; CD, Crohn's disease; HPRT, hypoxanthine guanine phosphoribosyltransferase; IBD, inflammatory bowel disease; MeTIMP, 6-methyl-thioinosine 5'-monophosphate; MTX, methotrexate; TPMT, thiopurine S-methyltransferase; UC, ulcerative colitis; XO, xanthine oxidase.

to those who did not, although the differences did not reach statistical significance. These findings suggested that patients with low XO expression might have a high risk of thiopurine-induced toxicity.

KEYWORDS
6-mercaptopurine, AZA thioprine, inflammatory bowel disease, xanthine oxidase

1 | INTRODUCTION

The thiopurines azathioprine (AZA) and 6-mercaptopurine (6-MP) have been widely used for induction and maintenance of remission in corticosteroid-dependent or corticosteroid-resistant inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), with a response rate of 55%–70%.[1] AZA is a prodrug with no immunosuppressive activity. The pharmacologic action is based on the formation of 6-MP, which is metabolized by hypoxanthine guanine phosphoribosyltransferase (HPRT) into an active class of metabolites known as 6-thioguaninenucleotides (6-TGNs). The other two major enzymes, xanthine oxidase (XO), and thiopurine methyltransferase (TPMT), are responsible for the conversion of 6-MP to the inactive metabolite 6-thiouric acid (6-TU) and methylated metabolites, including 6-methylmercaptopurine (6-MMP) and 6-methyl-thioinosine 5'-monophosphate (MeTIMP).[2]

The occurrence of adverse effects (AEs), such as leukopenia and hepatotoxicity, is a major drawback in the use of thiopurine drugs. Long-term thiopurine therapy fails in approximately 40% of patients who experience significant toxicity or inadequate response during the treatment.[3] The observed differences in therapeutic response or toxicity are partly explained by the variable formation of active metabolites due to genetic polymorphisms of the crucial enzymes in thiopurine metabolism. The enzyme TPMT could indirectly influence 6-TGN concentrations by shunting 6-MP metabolism away from 6-TGNs. Previous studies showed that TPMT deficiency was associated with severe, sometimes even fatal hematopoietic toxicity. Subjects with inherited TPMT deficiency presented higher levels of 6-TGNs and had an increased risk of myelotoxicity when they were treated with standard doses of thiopurines.[4,5]

However, not all adverse effects or metabolite patterns can be explained by genetic variations in TPMT. A previous study showed that only 27% of patients with CD and leukopenia had genetic variations of the TPMT gene associated with enzyme deficiency, whereas myelosuppression was more often caused by other factors.[6] The variation in concentration of thiopurine active metabolite and therapeutic outcomes could be a result of polymorphisms in all thiopurine-metabolizing enzymes. The relevance of enzymes other than TPMT to the clinical effects of these drugs has not been extensively evaluated.

XO is a cytoplasmic enzyme that catalyzes the last two steps of purine degradation: oxidation of hypoxanthine to xanthine and of xanthine to uric acid in human.[7] It catalyzes the catabolism of 6-MP, first to 8-hydroxy-6-mercaptopurine (8-OHMP) and subsequently to 6-TU.[8] The activity of XO is particularly high in the intestinal mucosa and liver, resulting in a substantial reduction of 6-MP bioavailability and thus lower levels of 6-TGNs.[9] The deficiencies (types I and II) of XO are rare autosomal recessive disorders with a combined incidence of about 1/70,000,[10] characterized by xanthinuria and xanthine lithiasis. Apart from these rare XO deficiencies, variations in human liver XO activity also exist, with approximately 20% of Caucasian participants displaying relatively lower enzyme activity than other ethnicities.[11] Population phenotyping studies using caffeine have indicated marked interindividual and interethnic differences in XO activity. Poor XO metabolizers comprise approximately 11% of the Japanese population, for example.[12] Furthermore, a sex-dependent difference in the level of hepatic XO activity in humans has also been indicated, with higher activity in male participants than in female participants.[13]

A previous case-report on XO showed a negative correlation between the XO activity and the concentration of 6-TGNs.[14] High variability of XO activity was found in Caucasian IBD patients, which was positively associated with male gender and the patient's age.[15] Complete XO deficiency has been shown to cause severe toxicity with full-dose AZA,[16] but the influence of the normal activity range of XO on the clinical efficacy of thiopurine therapy remains unknown. Therefore, the aims of this study were to investigate: (i) the distribution and variation of XO activity in Chinese IBD patients undergoing stable AZA/6-MP therapy and (ii) whether a relationship between XO activity and incidence of thiopurine-induced adverse effects (AEs) existed.

2 | MATERIALS AND METHODS

2.1 | Patients

A 3-year hospital-based retrospective investigation was performed to detect the activity of XO in thiopurine curative Chinese inflammatory bowel disease (IBD) patients. Consecutive patients with IBD diagnosis who received the AZA/6-MP treatment at the Gastroenterology Outpatient Clinic of the First Affiliated Hospital of Sun Yat-sen University were included in our study. The diagnoses of CD and UC were according to the criteria of Lennard-Jones,[17] based on clinical, endoscopic, histopathological, and radiological findings.

The location of the disease was determined according to Montreal Classification criteria.[18]

None of the patients had used any sulf-purine drugs previously, including AZA, 6-thioguanine (6-TG), and 6-MP. Patients with any of the following conditions were included in the study: steroid-dependent disease: unable to reduce corticosteroids below the equivalent of prednisolone 15 mg/day (or budesonide below 3 mg/day) within 3 months of starting corticosteroids; relapse within three months of stopping corticosteroids; frequent relapses: >3 relapses in one year or >2 relapses in 6 months; remission maintenance; or postoperative prophylaxis. Patients with any of the following conditions were excluded from the study: blood transfusion or administration of cyclosporine or methotrexate (MTX) within the last 3 months; treatments potentially interfering with AZA metabolism, including allopurinol and diuretics; insufficient function of heart, liver, or kidneys; any active infection; or pregnancy.

Drug dosages started with 1 mg/kg daily for AZA (Imuran, GlaxoSmithKline®) or 0.5 mg/kg daily for 6-MP (Purinethol, GlaxoSmithKline®) in the first week, then increased to 2 mg/kg daily for AZA and 1.0 mg/kg daily for 6-MP at the beginning of the second week and were held at this level until the end of the study.

Clinical data including sex, age, age at diagnosis, site of disease, type of IBD, weight, dose of AZA/6-MP, indication for AZA/6MP therapy, concomitant therapy (5-aminosalicylic acid (5-ASA), infliximab, or other drugs), and toxicity data—including full blood counts and liver function tests were collected and recorded by a clinician.

Control visits were performed every 2 weeks for the first month, every month for the following 2 months, and then every 3 months. During the visits, patients were given full blood counts and liver function tests, and they were clinically reviewed, with all AEs recorded. Hematotoxicity was diagnosed as leukopenia when the leukocyte count (WBC) was less than 3.5×10^9/L. When leukopenia was observed, the patient was removed from AZA/6-MP treatment and their WBC was continuously monitored for the following 2 days, with WBC recovery occurring over the next 1 to 2 weeks. Hepatotoxicity was defined as an increase in transaminase to at least triple the normal value. Pancreatitis was diagnosed when compatible symptoms (abdominal pain) were present and serum amylase was triple the normal upper limit. Flu-like symptoms included fever, headache, body aches, and arthralgia throughout the whole body. Gastrointestinal intolerance was defined as hypogeusia, nausea, and vomiting.

2.2 | Analysis of XO activity

For XO detection, 2 ml venous blood samples (EDTA anticoagulation) were obtained prior to treatment. Briefly, 2 ml of whole blood was centrifuged at 800*g* for 10 min at 4°C to isolate red cells. After washing the pellet twice with 2 ml of normal saline and centrifuging at 800*g* for 10 min, cells were gently resuspended in 2 ml of normal saline and the hematocrit was determined. The red cells were then lysed with 4°C ultrapure water (4 ml for 1 ml of solution) and centrifuged at 13,000*g* for 10 min at 4°C. The supernatant was kept at −80°C until analysis. Ultrapure water was obtained from a Milli-Q Plus water purification system (Millipore).

The XO activity in the red blood cells (RBC) of all patients was measured by a commercial kits (Xanthine Oxidase Assay Kit, A002-1-1), produced by Nanjing Jiancheng Bioengineering Institute®. The reaction of the kits was based on the oxidization of hypoxanthine to xanthine, which is catalyzed by XO. The reaction produces superoxide anion-free radicals, whereas the xanthine is forming. Combined with an electron acceptor and developer, it forms a compound detectable via UV spectrophotometry. This compound was used as a measure of XO activity, quantified with units of U/g Hb.

2.3 | Statistics

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, Version 16.0, IBM) and GraphPad Prism (Version 8.0.2, GraphPad Software). A one-sample Kolmogorov–Smirnov test was used to evaluate whether XO activity followed a normal (Gaussian) distribution. Quantitative variables were expressed as median and range, or as mean ± standard deviation when normally distributed. A parametric Student's *t*-test or nonparametric Mann–Whitney *U*-test was used to evaluate the differences between two independent groups. Analysis of covariance (ANCOVA) was used when factors such as age, gender, or combination with AZA were not matched between two groups. p less than .05 was considered statistically significant.

2.4 | Ethical considerations

The study was approved by the local Ethics Committee of Sun Yat-Sen University. Informed consent was obtained from all the patients before inclusion. The study was also registered on the International Standard Randomized Controlled Trial Number Register (ISRCTN) with trial number ISRCTN58287360.

3 | RESULTS

3.1 | Patient demographics

A total of 140 patients with IBD were included in this study. The selected baseline demographics and disease characteristics of the patients are shown in Table 1. All patients completed the 1-year follow-up visit after taking AZA/6-MP.

AEs were observed in 41 patients (29.3%) treated with AZA/6-MP. The most frequent one was leukopenia (26, 18.6%). One patient exhibited hepatotoxicity, and there were no occurrences of pancreatitis. The frequency of each adverse effect and corresponding medical decisions is summarized in Table 2.

3.2 | Distribution of XO activity

XO activity of 140 IBD patients ranged from 9.17 to 40.49 U/g Hb (20.29 ± 4.43 U/g Hb). As shown in Figure 1, the distribution of XO activity in IBD patients was normal-skew (Z = 0.675, p = .752). No deficient individual was found in this study.

No sex difference in XO activity was observed (p = .728), and the XO activity showed no difference between the UC and CD patients (p = .082) (Table 1).

TABLE 1 Baseline characteristics and XO activities of patients at time of inclusion

Characteristic	No. of patients (n = 140)	%	XO activity (U/g Hb)[a]	p value[b]
Sex				
Male	94	67.1	20.19 ± 4.75	.728
Female	46	32.9	20.47 ± 3.73	
Age(years)				
Median	33			
Range	3–74			
Diagnosis				
CD	38	27.1	20.68 ± 4.09	.082
UC	102	72.9	19.22 ± 5.15	
Prescription				
AZA	136	97.1	20.30 ± 4.47	.846
6-MP	4	2.9	19.86 ± 2.93	
Combination				
With 5-ASA	28	20.0	19.62 ± 5.07	.374
Without 5-ASA	112	80.0	20.45 ± 4.27	
With infliximab	11	7.9	19.41 ± 3.58	.497
Without infliximab	129	92.1	20.36 ± 4.50	

[a]Mean ± SD.; [b]Refer to the XO activity.

TABLE 2 Summary of adverse effects and corresponding medical decision in AZA/6-MP therapy

Adverse effects	No. of patients (n = 140)	Medical decision	
		Drug withdrawal	Other treatment
All[a]	41 (29.3%)	35 (81.4%)	
Leukopenia	26 (18.6%)	26 (100%)	
Hepatotoxicity	1 (0.7%)	0	Hepatinica
Flu-like symptoms	5 (3.6%)	4 (80%)	
Gastrointestinal intolerance	11 (7.9%)	6 (54.5%)	Digestant
Alopecia	5 (3.6%)	2 (40.0%)	

[a]Four patients had two or more adverse effects simultaneously.

3.3 | AEs and corresponding XO activity

There was a negative correlation between XO activity and the overall incidence of AEs (Table 3 and Figure 2). Mean XO activity was lower in patients with AEs than in those without AEs (18.40 ± 3.73 vs. 21.07 ± 4.48 U/g Hb, p = .001). This same trend held between patients with leukopenia and those without AEs (18.29 ± 3.68 U/g Hb vs. 21.07 ± 4.48 U/g Hb, p = .004). The XO activity difference between the patient with leukopenia and the patients without leukopenia was also significant (18.29 ± 3.68 vs. 20.74 ± 4.47 U/g Hb, p = .01). However, no significant difference in XO activity was found between patients with and without other AEs. Decreased XO activity was observed in the patients who developed flu-like symptoms (17.58 ± 3.50 U/g Hb) and alopecia (18.67 ± 2.91 U/g Hb) compared to those who did not, although the differences did not reach statistical significance.

4 | DISCUSSION

In many studies, differences in the therapeutic response to or toxicity of thiopurine drugs are partly explained by the variable formation of active metabolites due to genetic polymorphisms in the genes encoding crucial enzymes for thiopurine metabolism. The polymorphic enzyme TPMT plays an important role in thiopurine metabolism and the occurrence of adverse effects.[19,20] However, some studies have shown the disparity between TPMT's activity and its efficacy, even prior to AZA prescription.[21] This could be a result of different frequencies of TPMT genetic variation among subsets of the population and the combination of other drugs. Furthermore, TPMT is not the only risk factor that should be considered in thiopurine drug therapy. Our previous studies have indicated that the influence of other thiopurine metabolism enzymes is also important.[22–24]

XO is an early detoxifying enzyme in the metabolism of thiopurines, which oxidizes 6-MP to 6-thiouric acid, an inactive metabolite excreted in urine.[25] It is one of two major enzymes for the catabolism of thiopurines, yet its influence on metabolic response has not been studied extensively. XO is probably the most important enzyme determining thiopurine bioavailability—16%–72% for AZA and 5%–37% for 6-MP—and is responsible for its highly variable abundance. Generally, about two-thirds of a normal dose appears to be inactivated by the catabolic pathway of XO.[26] One case report on xanthine oxidase in patients with chronic autoimmune pancreatitis showed a negative correlation between XO activity and the concentration of 6-TGNs.[14] In another study, the authors found that XO activity was not related to Crohn's disease activity in 71 patients.[27] However, data on the correlation between XO activity and thiopurine-induced adverse effects is still deficient.

Blood XO level was usually used to reflect its liver lever in clinical laboratory diagnostics.[11] In this study, a negative correlation was found between XO activity and the occurrence of thiopurine-induced AEs. XO activity was lower in patients with AEs than in

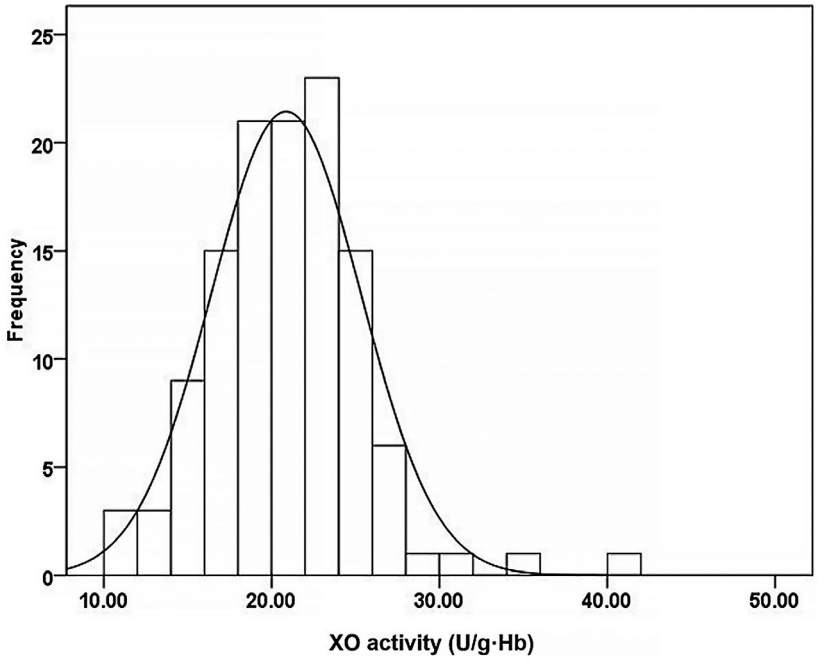

FIGURE 1 The distribution of XO activity in 140 IBD patients. XO activity of 140 IBD patients was 20.29 ± 4.43 U/g Hb. The distribution is normal-skew ($Z = 0.675$, $p = .752$)

TABLE 3 Adverse effects(AEs) and corresponding XO activity

	XO activity (U/g·Hb)[a]	*p* value[b]
Total	20.29 ± 4.43	
AEs	18.40 ± 3.73	.001
Leukopenia	18.29 ± 3.68	.004
Hepatotoxicity	13.36	
Flu-like symptoms	17.58 ± 3.50	.090
Gastrointestinal intolerance	20.17 ± 3.77	.524
Alopecia	18.41 ± 3.17	.193
Without AEs	21.07 ± 4.48	

[a]Mean ± SD.; [b]Versus without AEs.

those without AEs. Similarly, there was also a significant difference in XO activity between patients with leukopenia and without AEs. This offers a potential explanation for thiopurine toxicity in patients without TPMT polymorphisms.

It was reported that, in the hepatic tissues of 189 American patients undergoing clinically indicated partial hepatectomy or open liver biopsy, average XO activity was approximately 20% higher in male than in female patients.[11] Another study detected high variability of serum xanthine oxidoreductase activity in IBD patients[15]; however, the molecular basis of these differences remains unclear. Despite an observed four- to 10-fold variation between individuals in our study, which was consistent with previous reports, there was no observed difference in XO activity between the male and the female IBD patients. This may be the result of differences between study populations.

Classical xanthinuria, one of the inherited XO deficiencies, is a rare autosomal recessive disorder, and the combined incidence is reported to be 1 in 70,000.[10] In this study, no XO activity deficiency was observed, and no patient displayed symptoms of classical xanthinuria. Basal XO activity was detectable in all participants, all of whom were at an active stage of disease. It was impossible to evaluate the correlation between disease progression and XO activity, as all patients were at the same stage.

Until recently, the molecular basis of differences in XO activity has been unclear. It could be either genetically determined or influenced by environmental factors.[28] However, three single nucleotide polymorphisms (G514A, A3326C, and A3662G) in the XO gene can be used to categorize XO-deficient subjects as expressing at low, normal, or high levels, respectively.[7] Polymorphisms in the gene that encodes the XO enzyme have scarcely been studied in IBD patients. However, the influence of two single nucleotide polymorphisms (A1936G and A2107G) in the XO gene have been shown to have an effect on toxicity, hematological parameters, and thiopurine metabolite levels in patients with acute lymphoblastic leukemia (ALL) or IBD.[29] Of 35 IBD patients, three were heterozygous for both XO alleles, whereas only 1 of 19 ALL patients was heterozygous for both XO alleles. Genetic polymorphisms of XO and other factors that may also have an effect on the enzyme activity should be considered in future studies.

In our study, there were four patients who had two or more adverse effects simultaneously. Two of them had leukopenia accompanied by gastrointestinal intolerance. One had alopecia accompanied by leukopenia. The last had alopecia accompanied by gastrointestinal intolerance. One patient had three adverse effects simultaneously: leukopenia, flu-like symptoms, and gastrointestinal intolerance. There was no significant difference between XO activity in patients who experienced more than one adverse effect than those only experienced one (20.40 ± 2.45 U/g Hb vs. 18.12 ± 3.82 U/g Hb, $p = .206$). The XO activity of the patient who developed hepatotoxicity (13.36 U/g Hb) was much lower than the median of the other IBD

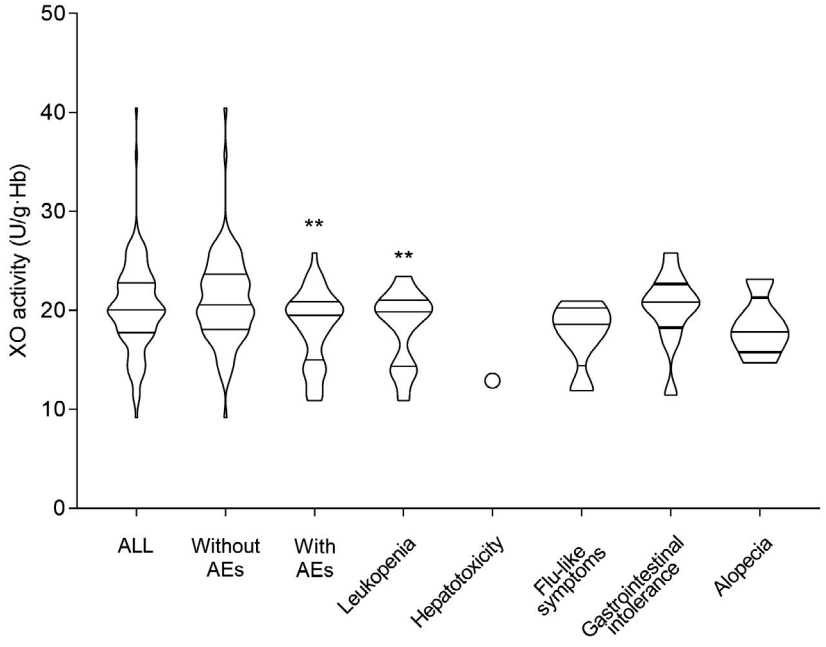

FIGURE 2 The distribution of XO activity in different adverse effects (AEs). ** Versus without AEs, $p < .01$. There was a negative correlation between XO activity and the overall incidence of AEs. Mean XO activity was lower in patients with AEs than in those without AEs (18.40c ± 3.73 vs. 21.07 ± 4.48 U/g Hb, $p = .001$). This same trend held between patients with leukopenia and those without AEs (18.29 ± 3.68 U/g Hb vs. 21.07 ± 4.48 U/g Hb, $p = .004$). However, no significant difference in XO activity was found between patients with and without other AEs. Decreased XO activity was observed in the patients who developed flu-like symptoms (17.58 ± 3.50 U/g Hb) and alopecia (18.67 ± 2.91 U/g Hb) compared to those who did not, although the differences did not reach statistical significance

patients. This finding suggests that the hepatotoxicity of thiopurine might be correlated with low XO activity, though further study with a larger sample size is needed.

In our study, of 28 patients who were taking AZA in combination with 5-ASA, 15 of them displayed AEs, of which 13 were leukopenia. In two separate retrospective studies of IBD patients, 5-ASA was shown to raise the concentration of 6-TGNs in patients' erythrocytes,[30,31] but the molecular mechanism of this observation should be studied further.

Our follow-up showed that, after drug withdrawal and being given symptomatic treatment, all 26 patients who developed leukopenia had returned to a normal blood cell count within 1–2 weeks. 17 of them were reintroduced to AZA (≤1 mg/kg daily, $n = 9$) or 6-MP (≤0.5 mg/kg daily, $n = 8$) and this reintervention was successful in 16 patients. One leukopenia patient was prescribed methotrexate (MTX), whereas four of them were shifted to infliximab. One patient experienced hepatotoxicity and transaminase levels were recovered within one month through hepatinica treatment without drug withdrawal. 11 patients exhibited gastrointestinal intolerance. Five of them recovered by taking digestants without drug withdrawal, whereas the other sixwere withdrawn from drug treatment, and the upset symptoms subsequently disappeared. Five patients developed flu-like symptoms, with four of them needing to discontinue therapy and only one remaining in treatment, as symptoms were mild. Five patients experienced alopecia, two of them severe enough to require discontinuation of therapy.

In conclusion, the results of this study show a negative correlation between the XO activity and the incidence of AZA/6-MP induced adverse effects, especially leukopenia. This finding gives us another perspective to explain toxicity associated with AZA/6-MP administration. Although this did not result in a prescriptive rule for all XO activity as a clinical index for thiopurine prescription, we suggest patients with liver disease not use thiopurine interventions, as their XO activity could be altered. If necessary, only low doses of thiopurine should be used by these patients, discreetly and with strict observation. There were some limitations of our study. We didn't detect the concentration of thiopurine inactive metabolites, which could better reflect the XO in vivo metabolomic activity. Moreover, the clinical impacts of other thiopurine metabolism enzymes on the toxic manifestations of thiopurine drug therapies still need to be elucidated in future studies.

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DISCLOSURE

All authors have no financial disclosures or conflicts of interest to declare.

AUTHORS' CONTRIBUTIONS

LD, FZ, and MH designed the study. LD, FZ, HL, and YZ collected the clinical samples and conducted the detections. FZ, XG, BC, and PH collected and organized the patients' information. HB, LH, and XW helped with the method development and data analysis. LD wrote the first draft of the paper, with revisions from HB, CL, PH, and MH. All authors contributed to revisions and approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Fraser AG, Orchard TR, Jewell DP. The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. *Gut*. 2002;50(4):485-489. https://doi.org/10.1136/gut.50.4.485
2. Lennard L. The clinical pharmacology of 6-mercaptopurine. *Eur J Clin Pharmacol*. 1992;43(4):329-339. https://doi.org/10.1007/BF02220605
3. Jharap B, Seinen ML, de Boer NK, et al. Thiopurine therapy in inflammatory bowel disease patients: analyses of two 8-year intercept cohorts. *Inflamm Bowel Dis*. 2010;16(9):1541-1549. https://doi.org/10.1002/ibd.21221
4. Gearry RB, Barclay ML. Azathioprine and 6-mercaptopurine pharmacogenetics and metabolite monitoring in inflammatory bowel disease. *J Gastroenterol Hepatol*. 2005;20(8):1149-1157. https://doi.org/10.1111/j.1440-1746.2005.03832.x
5. Sandborn WJ. A review of immune modifier therapy for inflammatory bowel disease: azathioprine, 6-mercaptopurine, cyclosporine, and methotrexate. *Am J Gastroenterol*. 1996;91(3):423-433.
6. Colombel J, Ferrari N, Debuysere H, et al. Genotypic analysis of thiopurine S-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. *Gastroenterology*. 2000;118(6):1025-1030. https://doi.org/10.1016/s0016-5085(00)70354-4
7. Kudo M, Moteki T, Sasaki T, et al. Functional characterization of human xanthine oxidase allelic variants. *Pharmacogenet Genomics*. 2008;18(3):243-251. https://doi.org/10.1097/FPC.0b013e3282f55e2e
8. Zimm S, Collins JM, O'Neill D, Chabner BA, Poplack DG. Inhibition of first-pass metabolism in cancer chemotherapy: interaction of 6-mercaptopurine and allopurinol. *Clin Pharmacol Ther*. 1983;34(6):810-817. https://doi.org/10.1038/clpt.1983.254
9. Parks DA, Granger DN. Xanthine oxidase: biochemistry, distribution and physiology. *Acta Physiol Scand Suppl*. 1986;548:87-99.
10. Harkness RA, McCreanor GM, Simpson D, MacFadyen IR. Pregnancy in and incidence of xanthine oxidase deficiency. *J Inherit Metab Dis*. 1986;9(4):407-408. https://doi.org/10.1007/BF01800499
11. Guerciolini R, Szumlanski C, Weinshilboum RM. Human liver xanthine oxidase: nature and extent of individual variation. *Clin Pharmacol Ther*. 1991;50(6):663-672. https://doi.org/10.1038/clpt.1991.205
12. Xu P, Hueckstedt TP, Harrison R, Hoidal JR. Molecular cloning, tissue expression of human xanthine dehydrogenase. *Biochem Biophys Res Commun*. 1994;199(2):998-1004. https://doi.org/10.1006/bbrc.1994.1328
13. Saruwatari J, Nakagawa K, Shindo J, et al. A population phenotyping study of three drug-metabolizing enzymes in Kyushu, Japan, with use of the caffeine test. *Clin Pharmacol Ther*. 2002;72(2):200-208. https://doi.org/10.1067/mcp.2002.126173
14. Wong DR, Derijks LJ, den Dulk MO, Gemmeke EH, Hooymans PM. The role of xanthine oxidase in thiopurine metabolism: a case report. *Ther Drug Monit*. 2007;29(6):845-848. https://doi.org/10.1097/FTD.0b013e31815bf4dc
15. Meijer B, Seinen ML, Hosman T, et al. High inter-individual variability of serum xanthine oxidoreductase activity in IBD patients. *Nucleosides Nucleotides Nucleic Acids*. 2018;37(6):317-323. https://doi.org/10.1080/15257770.2018.1460477
16. Serre-Debeauvais F, Bayle F, Amirou M, et al. Hematotoxicity caused by azathioprine genetically determined and aggravated by xanthine oxidase deficiency in a patient following renal transplantation. *Presse Med*. 1995;24(21):987-988.
17. Lennard-Jones JE. Classification of inflammatory bowel disease. *Scand J Gastroenterol*. 1989;170:2-6; discussion 16-9. https://doi.org/10.3109/00365528909091339
18. Silverberg MS, Satsangi J, Ahmad T, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol*. 2005;19(Suppl A):5A-36A. https://doi.org/10.1155/2005/269076
19. Ansari A, Arenas M, Greenfield SM, et al. Prospective evaluation of the pharmacogenetics of azathioprine in the treatment of inflammatory bowel disease. *Aliment Pharmacol Ther*. 2008;28(8):973-983. https://doi.org/10.1111/j.1365-2036.2008.03788.x
20. Reinshagen M, Schütz E, Armstrong VW, et al. 6-thioguanine nucleotide-adapted azathioprine therapy does not lead to higher remission rates than standard therapy in chronic active Crohn disease: results from a randomized, controlled, open trial. *Clin Chem*. 2007;53(7):1306-1314. https://doi.org/10.1373/clinchem.2007.086215
21. Gisbert JP, Luna M, Mate J, Gonzalez-Guijarro L, Cara C, Pajares JM. Choice of azathioprine or 6-mercaptopurine dose based on thiopurine methyltransferase (TPMT) activity to avoid myelosuppression. A prospective study. *Hepatogastroenterology*. 2006;53(69):399-404.
22. Ding L, Zhang F-B, Liu H, et al. Hypoxanthine guanine phosphoribosyltransferase activity is related to 6-thioguanine nucleotide concentrations and thiopurine-induced leukopenia in the treatment of inflammatory bowel disease. *Inflamm Bowel Dis*. 2012;18(1):63-73. https://doi.org/10.1002/ibd.21676
23. Fangbin Z, Xiang G, Liang D, et al. Prospective evaluation of pharmacogenomics and metabolite measurements upon azathioprine therapy in inflammatory bowel disease: an observational study. *Medicine (Baltimore)*. 2016;95(15):e3326. https://doi.org/10.1097/MD.0000000000003326
24. Liu H, Ding L, Zhang F, et al. The impact of glutathione S-transferase genotype and phenotype on the adverse drug reactions to azathioprine in patients with inflammatory bowel diseases. *J Pharmacol Sci*. 2015;129(2):95-100. https://doi.org/10.1016/j.jphs.2015.02.013

25. Sahasranaman S, Howard D, Roy S. Clinical pharmacology and pharmacogenetics of thiopurines. *Eur J Clin Pharmacol*. 2008;64(8):753-767. https://doi.org/10.1007/s00228-008-0478-6
26. Derijks LJ, Gilissen LP, Hooymans PM, Hommes DW. Review article: thiopurines in inflammatory bowel disease. *Aliment Pharmacol Ther*. 2006;24(5):715-729. https://doi.org/10.1111/j.1365-2036.2006.02980.x
27. Reuther LO, Sonne J, Larsen NE, et al. Pharmacological monitoring of azathioprine therapy. *Scand J Gastroenterol*. 2003;38(9):972-977. https://doi.org/10.1080/00365520310005082
28. Derijks LJ, Wong DR. Pharmacogenetics of thiopurines in inflammatory bowel disease. *Curr Pharm Des*. 2010;16(2):145-154. https://doi.org/10.2174/138161210790112773
29. Hawwa AF, Millership JS, Collier PS, et al. Pharmacogenomic studies of the anticancer and immunosuppressive thiopurines mercaptopurine and azathioprine. *Br J Clin Pharmacol*. 2008;66(4):517-528. https://doi.org/10.1111/j.1365-2125.2008.03248.x
30. de Boer NKH, Wong DR, Jharap B, et al. Dose-dependent influence of 5-aminosalicylates on thiopurine metabolism. *Am J Gastroenterol*. 2007;102(12):2747-2753. https://doi.org/10.1111/j.1572-0241.2007.01511.x
31. Hande S, Wilson-Rich N, Bousvaros A, et al. 5-aminosalicylate therapy is associated with higher 6-thioguanine levels in adults and children with inflammatory bowel disease in remission on 6-mercaptopurine or azathioprine. *Inflamm Bowel Dis*. 2006;12(4):251-257. https://doi.org/10.1097/01.MIB.0000206544.05661.9f

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