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[Original article]

Therapeutic Agents and Patient Characteristics Affecting Metabolism of Thiopurines in Patients with Inflammatory Bowel Disease

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ABSTRACT (196/200)

Objectives : In inflammatory bowel disease therapy, thiopurines have been essential. However, several reports have investigated factors affecting thiopurine metabolism to date. This study investigated factors affecting intracellular concentrations of 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine (6-MMP) in a real-world setting.

Methods : Between May 2013 and October 2021 in one institution, 44 patients (median age 44 years ; male 35, female 9 ; ulcerative colitis 32, Crohn's disease 12) receiving thiopurines were reviewed. Intracellular 6-TGN/6-MMP concentrations were measured by high-performance liquid chromatography, and the initial measurement in each patient was used for the study.

Results : The 6-TGN level was significantly higher in females, with mild disease activity, absence of NUDT15 polymorphism, and allopurinol administration. A higher trend was observed with high thiopurine dosage (>50 mg). 6-MMP levels were significantly lower with concomitant use of time-dependent 5-aminosalicylic acid (5-ASA) and allopurinol, and higher with high thiopurine dosage. On multivariate analysis of variance, logarithmically transformed 6-TGN levels were significantly higher in females, with high thiopurine dosage, and allopurinol administration. Similarly, logarithmically transformed 6-MMP levels were significantly higher with time-dependent 5-ASA administration and high thiopurine dosage.

Conclusions : Patients who received allopurinol, a high dose of thiopurine, or were female showed higher 6-TGN levels.

Key words : Thiopurine, 6-TGN, 6-MMP, inflammatory bowel disease

Introdution

In inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), a chronic inflammatory disorder of the intestinal mucosa causes repeated relapses and remissions, leading to the gradual accumulation of intestinal damage[1)]. Prompt induction of remission and its sustained maintenance are essential treatment strategies to eliminate intestinal damage[2,3)]. Recent advancements in IBD treatment have been significant, though thiopurines remain key drugs[4,5)]. Thiopurines were originally developed as therapeutic agents for childhood leukemia[6)], and their immuno-

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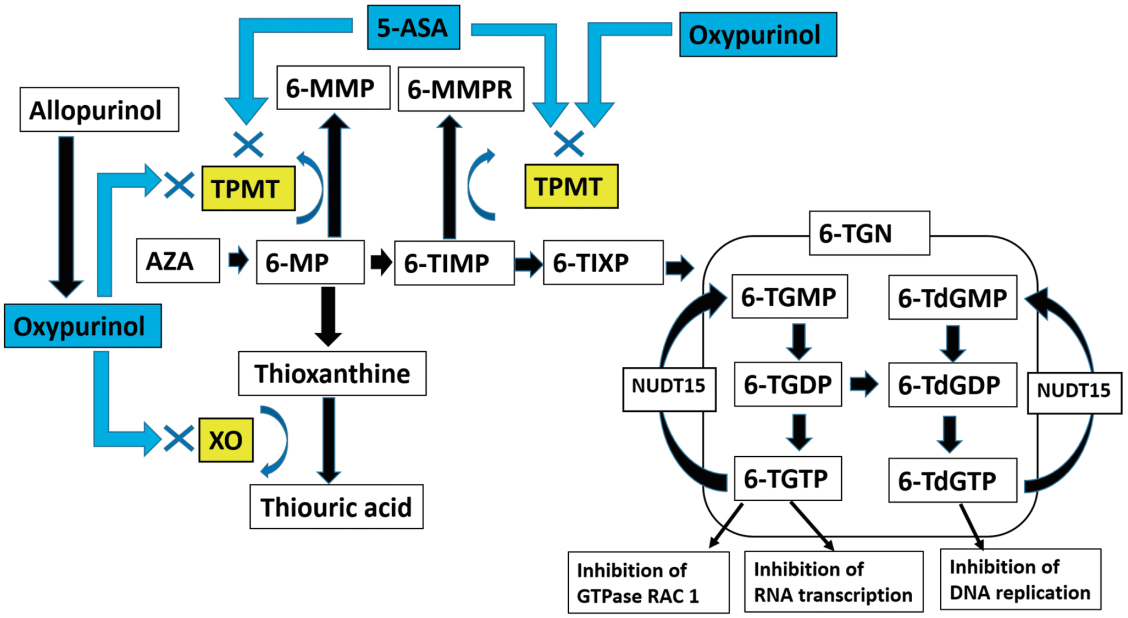

Fig. 1. Thiopurine metabolism. Metabolic enzymes are marked in yellow, and drugs related to them are marked in blue. 5-ASAs are thought to contribute to the increase in 6-TGN concentration by reducing 6-MMP by inhibiting TPMT activity. 6-MP has another pathway to be metabolized to thiouric acid via thioxanthine. Oxypurinol, a metabolite of allopurinol, causes elevation of thioxanthine by inhibiting xanthine oxidase (XO), which metabolizes thioxanthine. Oxypurinol also has an inhibitory effect on TPMT. Through these two mechanisms, allopurinol exerts a strong 6-TGN-increasing effect. AZA is non-enzymatically degraded to 6-MP and finally metabolized to 6-thioguanine nucleotide (6-TGN) via 6-thioinosine monophosphate (6-TIMP). 6-TGN inhibits intracellular nucleic acid synthesis and protein synthesis, thus suppressing the proliferation of activated lymphocytes. Thiopurine methyltransferase (TPMT) is an enzyme converting 6-MP to 6-methylmercaptopurine (6-MMP) and 6-TIMP to 6-methylmercaptopurine ribonucleotide (6-MMPR), and both metabolites (6-MMP and 6-MMPR) are associated with hepatotoxicity.

modulatory effects on IBD were initially reported in the late 1960s [7]. Currently, thiopurines are used to maintain long-term, steroid-free remission and to prevent secondary failure of anti-tumor necrosis factor α agents in IBD patients[8,9].

Several enzymes are involved in the metabolism of thiopurines, including azathioprine (AZA) and 6-mercaptopurine (6-MP), and these metabolic enzymes can be affected by various agents, as illustrated in Figure 1. It is well known that two key drugs used in the treatment of IBD patients affect thiopurine metabolism[10,11]. The first one is allopurinol, a uric acid synthesis inhibitor, which is metabolized to oxypurinol. Allopurinol exerts its effect on thiopurine metabolism by inhibiting both xanthine oxidase (XO) and thiopurine S-methyltransferase (TPMT)[12]. Through these two different pathways, allopurinol strongly increases the levels of 6-TGN, which is significantly and independently associated with the therapeutic response in IBD[13]. The second drug is 5-aminosalicylic acid (5-ASA), which interacts with thiopurine metabolism by inhibiting TPMT[14]. However, the effect of 5-ASA on thiopurine metabolism varies depending on the specific formulation, and the administration of time-dependent 5-ASA alone can impact thiopurine metabolism[15].

Apart from allopurinol and time-dependent 5-ASA, limited reports have examined the factors affecting the metabolism of thiopurines in real-world settings[16,17]. Furthermore, few reports have clarified the relationships between the concentrations of thiopurine metabolites and sex, age, NUDT15 (nucleoside diphosphate-linked moiety X-type motif 15), etc. This study focused on the intracellular concentrations of 6-TGN and 6-MMP associated with hepatoxicity and investigated the effects of concurrent medications and patient characteristics on the intracellular concentrations of 6-TGN and 6-MMP in IBD patients undergoing thiopurine therapy.

Methods

Study design

This was a single-center, retrospective, observational study. Before the study commencement, the Institutional Review Board of Fukushima Medi-

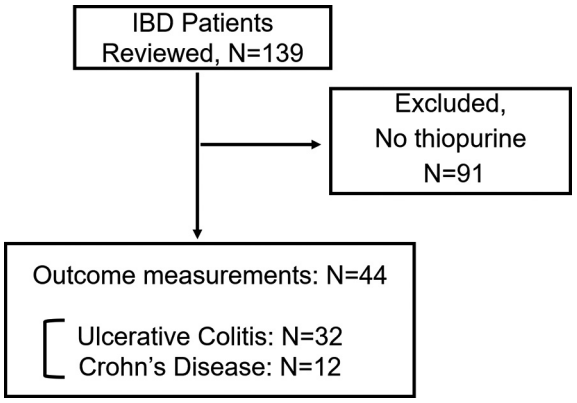

Fig. 2. Study flow of patients who received thiopurine treatment and underwent measurements of 6-TGN and 6-MMP concentrations.

cal University approved the study and waived the requirement for informed consent (Registration No. General 2021-212). All data were collected by December 2022. The Strengthening the Reporting of Observational Studies guidelines were followed in reporting this study.

Study Flow (Figure 2)

Of the 139 IBD patients who visited the Aizu Medical Center between May 2013 and October 2021, 44 patients (32 with UC, 12 with CD) who received thiopurine treatment and underwent 6-TGN and 6-MMP concentration measurements were included in the study (Figure 2). Relevant information, including short-term outcomes, was extracted from medical records. The intracellular concentrations of 6-TGN (therapeutic range : 235-450 pmol/8 $\times$ 10^8RBC) and 6-MMP (addiction level : $\geq$ 5,700 pmol/8 $\times$ 10^8RBC), were measured by the LSI Medience Corporation (Tokyo, Japan) using liquid chromatography- tandem mass spectrometry (LC-MS/MS). The 6-TGN concentrations in RBCs were measured Before starting thiopurine administration, the presence of NUDT15 polymorphism, which affects thiopurine metabolism and can lead to severe hematological toxicity in patients with the genetic variant, was primarily investigated. The dose of thiopurine was adjusted to a smaller dose, particularly at the time of initial prescription, to account for the risk of leukopenia in heterozygous patients. Disease activity was assessed using the partial-Mayo score (p-Mayo) for UC and the Crohn's Disease Activity Index (CDAI) for CD. Disease activity was categorized as "mild" (p-Mayo score $\leq$ 3, CDAI $\leq$ 220), "moderate" (4 $\leq$ p-Mayo score $\leq$ 6, 221 $\leq$ CDAI $\leq$ 450), or "severe" (p-Mayo score $>$ 6, CDAI $>$ 450). The dose of AZA (mg) in 6-MP was calculated with the conversion formula of 2.08 $\times$ 6-MP (mg)[18].

Outcome Measures

The initial measurement of 6-TGN and 6-MMP levels was conducted 3$\pm$1 months after initiating thiopurine administration, and the initial measurement of each patient was used for this study. The primary outcome measure was to investigate factors affecting 6-TGN levels, including patient background factors such as age, sex, UC or CD diagnosis, disease activity, and NUDT15 polymorphism, as well as concomitant therapeutic agents (thiopurine dose and formulation, 5-ASA dose and formulation, steroids, biologics, and allopurinol). The secondary outcome measure was to investigate factors affecting 6-MMP levels and adverse events. In addition, the 5-ASA formulation was categorized into time-dependent 5-ASA and other types of 5-ASA (pH-dependent, multimatrix, salazosulfapyridine) and investigated the effect of time-dependent 5-ASA on 6-TGN and 6-MMP levels, because time-dependent 5-ASA can be absorbed in the small intestine and inhibit TMPT activity in the blood.

Statistical Methods

Patients' characteristics are reported as descriptive statistics, with continuous variables expressed as median and interquartile range (IQR) values and categorical variables expressed as counts and percentages. Continuous variables were compared using the Mann-Whitney U test, and categorical variables were compared by Fisher's exact test. Furthermore, a one-way analysis of variance (ANOVA) was performed, taking into consideration the normal distribution exhibited by the logarithmically transformed 6-TGN/6-MMP levels. Subsequently, multivariate ANOVA was performed, incorporating variables with a significance level set at $p<0.2$. The normality of the distribution was assessed by the Shapiro-Wilk W test. All p values are two-tailed, and values <0.05 were considered significant. All statistical analyses were performed with Stata 16.1 (Stata Corp, College Station, Texas, USA).

Results

Patients' Characteristics (Table 1)

A total of 44 IBD patients (35 males, 9 females), with a median age of 44 years, were included in the study. Of these patients, 32 had UC and 12 had CD. NUDT15 polymorphism was measured in 40, and

Table 1. Patients' characteristics

		N=44
Age, y	Median [interquartile range]	44 [31–57]
Sex, *n* (%)	Male	35 (80%)
	Female	9 (20%)
Disease, *n* (%)	Ulcerative colitis	32 (72%)
	Crohn's disease	12 (28%)
NUDT15 (n=40)*, *n* (%)	Arg/Arg	36 (90%)
	Arg/Cys	4 (10%)
	Cys/Cys	0 (0%)
Disease activity, *n* (%)	Moderate	8 (18%)
	Mild	36 (82%)
Thiopurine, *n* (%)	Azathioprine	39 (89%)
	6-mercaptprine	5 (11%)
Concomitant drug**, *n* (%)	Corticosteroids	11 (25%)
	5-ASA	38 (86%)
	Time-dependent 5-ASA	17 (39%)
	Biologics	17 (39%)
	Allopurinol	16 (36%)
Severe adverse events	Occurrence	0 (0%)

NUDT15 : Nucleoside diphosphate-linked moiety X-type motif 15, 5-ASA : 5-aminosalicylic
* Four patients did not undergo investigation of NUDT15 polymorphism
** Overlapping cases are involved.

the NUDT15 variants showed a prevalence of 10%, with 4 patients having the Arg/Cys variant and none having the Cys/Cys variant. Regarding disease activity, 82% of patients had mild activity, 18% had moderate activity, and none had severe activity. In addition, all patients with CD showed mild activity. Most patients underwent administration of AZA (89%) as thiopurine and 5-ASA (86%) as concomitant drugs. No severe adverse events, including severe hematological toxicity and alopecia, were observed during the study period.

Factors affecting 6-TGN and 6-MMP levels

As shown in Table 2A, the 6-TGN levels showed significant differences by sex (p=0.019), disease activity (p=0.048), and NUDT15 polymorphism (p=0.027). In terms of concomitant agents, there was a significant increase in patients who were administered allopurinol (p=0.001) and a trend toward an increase in patients receiving high-dose thiopurine treatment (p=0.104).

The 6-MMP levels did not show any significant differences in patients' characteristics, as shown in Table 2B. Nonetheless, significant increases were observed in patients who underwent high-dose thiopurine treatment (p=0.0004) and in those who did not receive time-dependent 5-ASA (p=0.0052) or allopurinol (p=0.036).

Effect of 5-ASA Formulations on 6-TGN and 6-MMP Levels

The levels of 6-TGN were 300 [interquartile range 257–356] pmol/8 × 10^8 RBC in the 5-ASA-free group, 362 [237–518] pmol/8 × 10^8 RBC in the time-dependent 5-ASA group, and 272 [185–463] pmol/8 × 10^8 RBC in the other types of 5-ASA group, as shown in Figure 3(a). However, no significant differences were observed in any of the comparisons made. In contrast, 6-MMP levels were 369 [170–501] pmol/8 × 10^8 RBC in the 5-ASA-free group, 131 [89–202] pmol/8 × 10^8 RBC in the time-dependent 5-ASA group, and 330 [141–470] pmol/8 × 10^8 RBC in the other types of 5-ASA group. Significant differences were found between the time-dependent 5-ASA group and the 5-ASA-free group (p=0.035), as well as between the time-dependent 5-ASA group and the other types of 5-ASA group (p=0.011), as shown in Figure 3(b).

Factors Affecting Logarithmically Transformed 6-TGN and 6-MMP Levels

The 6-TNG levels followed a normal distribution after logarithmic transformation (Shapiro-Wilk W test, p = 0.938). One-way ANOVA identified significant differences by sex (p = 0.0141) and administration of allopurinol (p = 0.0035). Factors

Table 2A. Concentration of 6-TGN by factor (pmol/8×10^8 RBC)

	Yes	No	*P* value*
Age (Yes : >40 y n=27, No : ≤39 y n=17)	328 [237-517]	340 [129-495]	0.46
Sex (Yes : male n=35, No : female n=9)	272 [185-447]	518 [341-790]	0.019
Disease (Yes : CD n=12, No : UC n=32)	383 [251-619]	282 [215-455]	0.38
Disease activity (Yes : mild n=36, No : moderate n=8)	353 [247-518]	215 [138-255]	0.048
NUDT15 (Yes : Arg/Arg n=36, No : Arg/Cys n=4)**	345 [245-528]	163 [112-230]	0.027
Dose of thiopurine (Yes : >50 mg n=25, No : ≤50 mg n=19)	341 [257-463]	256 [123-518]	0.104
5-ASA (Yes : n=38, No : n=6)	341 [203-517]	300 [257-356]	0.84
Time-dependent 5-ASA (Yes : n=17, No : n=27)	362 [237-518]	291 [123-463]	0.406
Corticosteroid (Yes : n=11, No : n=33)	261 [227-349]	356 [203-518]	0.25
Biologics (Yes : n=17, No : n=27)	272 [152-790]	340 [227-463]	0.82
Allopurinol (Yes : n=16, No : n=28)	506 [359-745]	255 [169-345]	0.001

Values are expressed as median [interquartile range] values.
* Mann-Whitney U test
** Four patients did not undergo investigation of NUDT15 polymorphism
6-TGN : 6-thioguanine, CD : Crohn's disease, NUDT15 : Nucleoside diphosphate-linked moiety X-type motif 15, 5-ASA : 5-aminosalicylic acid

Table 2B. Concentration of 6-MMP by factor (pmol/8×10^8 RBC)

	Yes	No	*P* value*
Age (>40 y : n=27)	202 [97-470]	230 [131-350]	0.75
Sex (male : n=35)	230 [97-418]	145 [124-330]	0.57
Disease (CD : n=12)	264 [118-400]	159 [100-412]	0.72
Disease activity (mild : n=36)	161 [93-425]	312 [177-412]	0.47
NUDT15 (Arg/Arg : n=36)**	186 [100-401]	454 [245-658]	0.22
Dose of thiopurine (≥50 mg : n=25)	350 [145-501]	102 [51-203]	0.0004
5-ASA (yes : n=38)	177 [97-406]	369 [170-501]	0.21
Time-dependent 5-ASA (yes : n=17)	131 [89-202]	333 [141-501]	0.0052
Corticosteroid (yes : n=11)	230 [97-404]	202 [108-446]	0.92
Biologics (yes : n=17)	170 [84-350]	203 [102-446]	0.52
Allopurinol (yes : n=16)	144 [59-268]	291 [128-474]	0.036

Values are expressed as median [interquartile range] values.
* Mann-Whitney U test
** Four patients did not undergo investigation of NUDT15 polymorphism
6-MMP : 6-methyl mercaptopurine, CD : Crohn's disease, NUDT15 : Nucleoside diphosphate-linked moiety X-type motif 15, 5-ASA : 5-aminosalicylic acid

such as disease activity (p = 0.189), NUDT15 polymorphism (p = 0.0503), and thiopurine dose (p = 0.0529) were associated with 6-TGN levels. On multivariate ANOVA with these covariates, sex (p = 0.0052), thiopurine dose (p = 0.0015), and administration of allopurinol (p = 0.0003) showed significant associations with the logarithmically transformed 6-TGN level, as shown in Table 3A.

Similarly, the logarithmically transformed 6-MMP levels showed a normal distribution (Shap-

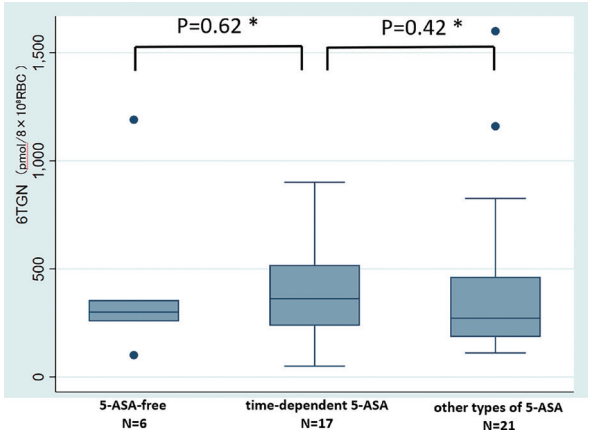

Fig. 3(a)

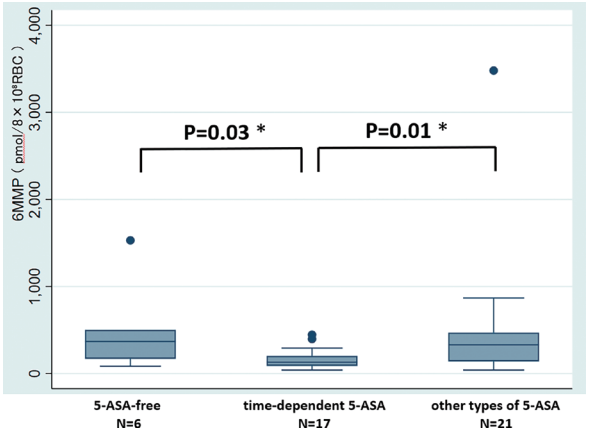

Fig. 3(b)

Fig. 3. Effects of 5-ASA formulations (5-ASA-free, time-dependent 5-ASA, other types of 5-ASA) on 6-TGN (a) and 6-MMP (b) concentrations. Significant differences are seen between the time-dependent 5-ASA group and the 5-ASA-free group, as well as between the time-dependent 5-ASA group and the other types of 5-ASA group, in 6-MMP concentrations. The Mann-Whitney U test was used for comparisons.

Table 3A. Factors Affecting the Logarithmically Transformed 6-TGN Level

	ln (6-TGN Level)		*P* value	
	Yes	No	One-way ANOVA	Multivariate ANOVA
Age (>40 y : n=27)	5.87 ± 0.63	5.63 ± 0.87	0.294	-
Sex (male : n=35)	5.64 ± 0.71	6.30 ± 0.60	0.014	0.0052
Disease (CD : n=12)	5.57 ± 0.90	5.86 ± 0.66	0.248	-
Disease activity (mild : n=36)	5.85 ± 0.70	5.47 ± 0.84	0.189	0.709
NUDT15 (Arg/Arg : n=36)	5.85 ± 0.75	5.07 ± 0.43	0.050	0.417
Dose of thiopurine (≥50 mg : n=25)	5.96 ± 0.58	5.53 ± 0.85	0.053	0.0015
5-ASA (yes : n=38)	5.78 ± 0.87	5.75 ± 0.79	0.935	-
Time-dependent 5-ASA (yes : n=17)	5.83 ± 0.72	5.75 ± 0.75	0.738	-
Corticosteroid (yes : n=11)	5.60 ± 0.57	5.84 ± 0.78	0.367	-
Biologics (yes : n=17)	5.75 ± 0.96	5.80 ± 0.56	0.844	-
Allopurinol (yes : n=16)	6.19 ± 0.58	5.54 ± 0.71	0.0035	0.0003

"ln (x)" means the natural logarithm of X. ANOVA : analysis of variance, NUDT15 : Nucleoside diphosphate-linked moiety X-type motif 15, 5-ASA : 5-aminosalicylic acid

iro-Wilk W test, p = 0.348). One-way ANOVA identified significant differences based on thiopurine dose (p = 0.0002), time-dependent 5-ASA (p = 0.0068), and allopurinol (p = 0.024). On multivariate ANOVA with those covariates, only the thiopurine dose showed a significant difference (p = 0.0015), as shown in Table 3B.

Discussion

This study investigated factors affecting intracellular 6-TGN and 6-MMP levels in IBD patients

Table 3B. Factors Affecting the Logarithmically Transformed 6-MMP Level

	ln (6-MMP Level)		*P* value	
	Yes	No	One-way ANOVA	Multivariate ANOVA
Age (>40 y : *n*=27)	5.39 ± 1.13	5.23 ± 0.85	0.627	-
Sex (male : *n*=35)	5.37 ± 1.06	5.17 ± 0.88	0.608	-
Disease (CD : *n*=12)	5.35 ± 0.82	5.32 ± 1.10	0.919	-
Disease activity (mild : *n*=36)	5.28 ± 1.05	5.52 ± 0.91	0.556	-
NUDT15 (Arg/Arg : *n*=36)	5.29 ± 1.03	5.84 ± 0.98	0.319	-
Dose of thiopurine (≥50 mg : *n*=25)	5.79 ± 0.96	4.71 ± 0.76	0.0002	0.0015
5-ASA (yes : *n*=38)	5.25 ± 1.02	5.82 ± 0.99	0.206	-
Time-dependent 5-ASA (yes : *n*=17)	4.81 ± 0.73	5.65 ± 1.06	0.0068	0.0182
Corticosteroid (yes : *n*=11)	5.34 ± 0.97	5.32 ± 1.05	0.9567	-
Biologics (yes : *n*=17)	5.14 ± 0.88	5.44 ± 1.10	0.3504	-
Allopurinol (yes : *n*=16)	4.87 ± 0.88	5.59 ± 1.02	0.024	0.226

"ln (x)" means the natural logarithm of X. ANOVA : analysis of variance, NUDT15 : Nucleoside diphosphate-linked moiety X-type motif 15, 5-ASA : 5-aminosalicylic acid

and confirmed the effects of allopurinol, thiopurine dosage, and time-dependent 5-ASA in a real-world setting. Administration of corticosteroids and biologics did not show any significant effects on 6-TGN and 6-MMP levels. As for patient factors, 6-TGN levels were affected by sex, disease activity, and status of NUDT15 polymorphism on univariate analysis, and sex alone significantly affected 6-TGN levels on multivariate ANOVA. In contrast, no patient factors affected 6-MMP levels.

Administration of allopurinol had a significant effect on 6-TGN and 6-MMP levels, except on multivariate ANOVA. This may be attributed to the double inhibitory action on XO and TPMT[12]. This action reduces hepatotoxicity caused by 6-MMP and can also enhance the efficacy of thiopurine by increasing 6-TGN levels, even with a small amount of thiopurine. In fact, the therapeutic efficacy for mucosal healing and safety of low-dose thiopurine in combination with allopurinol has been reported in recent years[19].

The dose of thiopurine affected both 6-TGN and 6-MMP levels, with higher 6-TGN levels observed in females. Since women generally tend to have smaller physiques than men, this observation may seem obvious. Nevertheless, clinicians should still be aware of these findings when prescribing thiopurine. In addition, 6-MMP can be greatly affected by thiopurine dose because 6-MMP is generated in the early phase of thiopurine metabolism.

Previous reports demonstrated that 6-TGN levels increased significantly in CD patients who received thiopurine with 5-ASA compared to those who did not[14]. Morikubo *et al.* examined 6-TGN levels with different 5-ASA types and reported a significant concentration increase of 6-TGN with time-dependent 5-ASA. This mechanism is thought to involve the fact that a large proportion of time-dependent 5-ASA is absorbed in the epithelium of the small intestine and inhibits TPMT activity[15]. In the present study, however, administration of time-dependent 5-ASA decreased 6-MMP levels, but it did not significantly increase 6-TGN levels. This inconsistency may be related to less statistical power due to the small sample size.

As for patient background, a significant increase in 6-TGN levels was observed in patients with mild disease activity compared with moderate cases. This suggests that disease activity could be controlled in patients with high 6-TGN levels. Indeed, Hanai *et al.* reported that 6-TGN levels in UC patients were significantly lower in the relapse

group[20]. Generally, thiopurine is not used in both UC and CD with severe disease activity because it takes approximately two months for the effect of thiopurine to manifest. In addition, no patients with severe disease activity received thiopurine in the present study.

NUDT15 is a gene involved in the metabolism of 6-TGN. One percent of Asians have a homozygous gene mutation, and severe side effects such as severe leukopenia and alopecia can be seen[21]. Therefore, the identification of NUDT15 genetic mutations is essential for Asian patients. Theoretically, its genetic polymorphism does not change the total amount of 6-TGN, but it alters the proportion of 6-TGMP (6-thio-guanosine monophosphate), 6-TGDP (6-thio-guanosine diphosphate), and 6-TGTP (6-thio-guanosine triphosphate), all in the 6-TGN family[22]. In the present study, 6-TGN concentrations were lower in heterozygous patients. A plausible explanation is that the dose of thiopurine was lowered to a smaller dose, particularly at the time of initial prescription, to account for the risk of leukopenia in heterozygous patients, although this tendency was not clear in the present study.

This study has several limitations. First, this was a retrospective study conducted in a single hospital and subject to selection bias. Second, the number of patients examined was relatively small. With a small sample size, the possibility of committing a Type II error (β), which is judged as no significant difference when in fact there is a significant difference, must always be considered. Indeed, the administration of time-dependent ASA did not affect 6-TGN levels in this study. Third, only 6-TGN and 6-MMP levels were measured of the various thiopurine metabolites. For instance, 6-MMPR (6-methylmercaptopurine ribonucleotide), which is generated during the metabolic process of 6-MP and may cause liver damage, was not at all investigated. Other thiopurine metabolites might be measured to further elucidate thiopurine metabolism in a future study.

In conclusion, patients who received allopurinol, a high dose of thiopurine, or were female exhibited higher 6-TGN levels in a real-world setting. Patients who received high-dose thiopurine had higher 6-MMP levels, but those given allopurinol or time-dependent 5-ASA had lower 6-MMP levels. Clinicians should take note of these findings that 6-TGN and 6-MMP concentrations were affected by patient background factors, including NUDT15 gene polymorphism, and concomitant drugs when prescribing thiopurine.

Conflict of Interest

The authors have no competing interests to disclose.

Acknowledgment

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Ethics approval and consent to participate

Approval for a waiver of informed consent was obtained from the Institutional Review Board of Fukushima Medical University (Registration No. 2021-212).

Authors' Contributions

Masato Aizawa and Kazutomo Togashi wrote the main document. Masato Aizawa, Kazutomo Togashi, Kohei Suzuki, Yuki Nakajima and Kenichi Utano substantially contributed to the conception of the work. Masato Aizawa, Kazutomo Togashi, Kohei Suzuki, Yuki Nakajima and Kenichi Utano, Kana Tamazawa, Kenta Ueda, Jun Wada, Kentaro Sato, and Goro Shibukawa contributed to the acquisition of data. Masato Aizawa, Kazutomo Togashi and Noriko Suzuki contributed to the interpretation of data. All authors reviewed the manuscript.

Data Availability

The raw data used in this study are available upon request from the corresponding author for replication and verification.

Abbreviations

IBD : inflammatory bowel disease
UC : ulcerative colitis
CD : Crohn's disease
5-ASA : 5-aminosalicylic acid
AZA : azathioprine
6-MP : 6-mercaptopurine
6-TGN : 6-thioguanine nucleotide
6-TIMP : 6-thio-inosine monophosphate
6-TXMP : 6-thio-xanthosine monophosphate
6-MMP : 6-methylmercaptopurine
6-MMPR : 6-methylmercaptopurine ribonucleotide
6-TGMP : 6-thio-guanosine monophosphate
6-TGDP : 6-thio-guanosine diphosphate

6-TGTP : 6-thio-guanosine triphosphate
6-TdGMP : 6-thio-deoxyguanosine monophosphate
6-TdGDP : 6-thio-deoxyguanosine diphosphate
6-TdGTP : 6-thio-deoxyguanosine triphosphate
TPMT : Thiopurine S-methyltransferase
NUDT15 : Nucleoside diphosphate-linked moiety X-type motif 15
p-Mayo : partial-Mayo score
CDAI : Crohn's disease activity index
IQR : interquartile rage
ANOVA : analysis of variance
XO : xanthine oxidase

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