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Lower doses of 6-mercaptopurine/azathioprine bring enough clinical efficacy and therapeutic concentration of erythrocyte 6-mercaptopurine metabolite in Japanese IBD patients

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Abstract

Background: Although 6-mercaptopurine (6-MP) and azathioprine (AZA) are prescribed at lower doses, their efficacy in patients with inflammatory bowel disease (IBD) in Japan is comparable to that in Europe/America. However, there has been no report concerning the measurement of erythrocyte 6-thioguanine nucleotides (6-TGN), which is an active metabolite of 6-MP or AZA, in Japanese IBD patients. This study was designed to elucidate the pharmacokinetic–pharmacodynamic properties of 6-MP and AZA in Japanese patients by measurement of erythrocyte 6-TGN level. *Methods*: 134 adult patients (99 males; 35 females) with IBD (75 ulcerative colitis; 59 Crohn's disease) who had been receiving a constant dose of 6-MP or AZA for three months or longer were enrolled. Erythrocyte 6-TGN levels were measured using the low-pressure gradient HPLC method, and correlated with treatment efficacy. The genetic polymorphism of thiopurine methyltransferase (TPMT) genotype was also assessed.

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Results: The mean erythrocyte 6-TGN level (mean ± SD) was 342.3 ± 220.9 pmol/8 × 10^8RBC, which was supposed to be therapeutic concentration, although the mean daily doses of 6-MP and AZA were no more than 29.8 ± 9.9 mg/day of 6-MP equivalent. However, all patients were identified with the wild type of TPMT genotype. There was no significant difference in the mean 6-TGN levels between patients in remission and no-remission group. The mean 6-TGN level was significantly higher in the once-daily administration group than three times-daily group.
Conclusion: Thirty mg/day of 6-MP or 50 mg/day of AZA, once-daily oral administration in Japanese IBD patients was sufficient to achieve the therapeutic target level of 6-TGN in Europeans/Americans.

1. Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are refractory chronic inflammatory bowel diseases (IBD). The cause and pathogenesis are unclear, and the therapeutic target is to improve the patient's QOL by maintaining the clinical remission of via long-term medication.

In drug therapy in Japan, 5-amynosalicylic acid (5-ASA) preparations, salazosulfapyridine and mesalazine, and adrenocortical steroid, prednisolone (PSL), are basically administered, but fewer effective treatments are available for cases with difficulty in steroid withdrawal and steroid dependence, in which a dose reduction of PSL induces recurrence or aggravation, and the adverse effects of long-term steroid treatment have been an issue. Immunosuppressants, 6-mercaptopurine (6-MP), and its prodrug, azathioprine (AZA), have been reported to allow the dose reduction of PSL in cases with difficulty in steroid withdrawal and steroid dependence, and maintain remission in Europeans/Americans,[1,2] and 6-MP (10% powder) or AZA (50 mg tablet) has also come to be applied in IBD cases with difficulty in PSL dose reduction and withdrawal in Japan.

Neither 6-MP nor AZA is active in their unchanged forms. They are absorbed from the gastrointestinal tract and metabolized to 6-thioinosine nucleotides by hypoxanthine phosphoribosyl transferase (HPRT) in cells, and finally to 6-thioguanine nucleotides (6-TGN). Since 6-TGN is structurally very similar to the purine base, it is incorporated into cellular DNA as a deceptive base, and exhibits a pharmacological effect.[3]

A close association of the erythrocyte levels of 6-TGN and a non-active metabolite, 6-methylmercaptopurine (6-MMP), with the therapeutic or adverse effect has recently been reported.[4,5]

In European/American reports, 6-MP was generally administered at 1.5 mg/kg body weight/day and AZA at 2.0–3.0 mg/kg body weight/day,[6] but half doses per body weight: 30 mg/day 6-MP and 50 mg/day AZA, have been reported to be effective in Japanese.[7,8]

However, the appropriateness of the dosage for IBD applied in Japan based on clinical experience: 30 mg/day 6-MP and 50 mg/day AZA, and equivalence between the uses of 6-MP and its prodrug, AZA, have not yet been investigated in Japanese IBD patients with regard to the 6-TGN and 6-MMP levels. Moreover, there has been no report in which PSL dose reduction and maintenance of remission by 6-MP and AZA were evaluated with regard to the relationship with the 6-TGN levels.

In this study, we investigated the safe and adequate dosage of 6-MP or AZA for Japanese IBD patients using the erythrocyte 6-MP metabolite (6-TGN and 6-methylmercaptopurine ribonucleotides (6-MMPR)) concentrations. We also investigated the influence of dosing frequency and dosage form of 6-MP and AZA on the erythrocyte 6-TGN levels.

The study was approved by the Ethics Committee of Keio University Hospital and Kitasato Institute Hospital and performed in patients who gave written consent to participate in the study.

2. Materials and methods

2.1. Subjects

The subjects were 134 in- and outpatients with IBD (UC: 75, CD: 59) being treated with 6-MP or AZA at a constant dose for 3 months or longer at Keio University Hospital or Kitasato Institute Hospital (Table 1).

Patients who had been treated with other immunosuppressants, such as cyclosporine, tacrolimus, and methotrexate, within 1 month before measurement of the erythrocyte 6-MP metabolite concentrations, those who had undergone leucocytopheresis (LCAP), granulocytopheresis (GCAP), and those who had been treated with a chimeric anti-human TNF α monoclonal antibody, infliximab, within 2 months before the measurement were excluded.

Table 1 Characteristics of IBD patients treated with 6-MP or AZA

Gender (M/F)	(*n*)	99/35
Age (mean ± SD)	(year)	38.0 ± 12.0
Disease classification (UC/CD)	(*n*)	75/59
6-MP treatment/AZA treatment	(*n*)	112/22
Duration of treatment (mean ± SD)	(month)	39.5 ± 39.0
6-MP	(month)	43.2 ± 41.3
AZA	(month)	21.1 ± 15.0
6-MP dose (mean ± SD)	(mg/day)	29.8 ± 9.9
6-MP	(mg/day)	29.9 ± 9.7
AZA (convert into 6MP)	(mg/day)	29.5 ± 11.8
Patient with 5-ASA treatment	(*n*)	127
5-ASA dose (mean ± SD)	(g/day)	2.1 ± 0.7
UC	(g/day)	1.7 ± 0.6
CD	(g/day)	2.4 ± 0.9

The oral dose of AZA, was converted to the 6-MP oral dose equivalent by dividing by 2.08 based on the molecular weight and in-vivo conversion rate.[9]

2.2. Measurements of the erythrocyte 6-MP metabolites levels

For measurement of the erythrocyte 6-TGN and 6-MMPR levels, the low-pressure gradient HPLC method reported by Mawatari et al.[10] was partially modified and used.

Heparinized blood (10 ml) was collected at the time of visiting the outpatient clinic or during hospitalization. Red blood cells were separated by centrifugation, washed with physiological saline, and stored at −80 °C until measurement. On calculating the erythrocyte 6-MP metabolite levels, the red blood cell count was corrected with the erythrocyte hemoglobin level, and the metabolite levels per unit red blood cell count (8×108 cells) were calculated.

2.3. Determination of thiopurine *S*-methyltransferase (TPMT) genotype

Genomic DNA was extracted from whole blood using a QIAamp® DNA Mini Kit (QIAGEN,Tokyo). Genotyping assays were carried out by polymerase chain reaction (PCR)-restriction fragment length polymorphism/allele-specific PCR according to the procedure reported by Ishioka et al.[11] with minor modifications.

All 134 subjects were genotyped at G460A (TPMT*3A and TPMT*3B), A719G (TPMT*3A and TPMT*3C), and G238C (TPMT*2), which contain the 2 most common variant nucleotide sequences in individuals with low TPMT activity. Alleles without any of the assayed mutations were assumed to be the TPMT wild-type gene (TPMT*1).

2.4. Clinical activity

Patient clinical information was collected from the medical records filled in by physicians and laboratory test records, and the activity index at the time of 6-MP metabolite measurement was calculated using the Clinical Activity Index for the Evaluation of Patients with Ulcerative Colitis (CAI) for UC,[12] and Crohn's Disease Activity Index (CDAI) for CD.[13] Patients with a CAI of 4 or lower and CDAI of 150 or lower were regarded as in remission.

2.5. Steroid dose reduction

The PSL doses (mg/day) at the times of treatment initiation and 6-MP metabolite measurement were compared in 76 patients treated with 6-MP or AZA for PSL dose reduction. Patients under the glucocorticoid pulse therapy at the time of 6-MP or AZA treatment initiation were excluded.

2.6. Influence of differences in dosing frequency

In patients who orally administered 30 mg/day 6-MP or 50 mg/day AZA (24.0 mg as 6-MP), the influence of differences in the dosing frequency: once a day (6-MP or AZA) and 3 times a day (6-MP), on the 6-TGN level was investigated.

2.7. Influence of differences in drug and dosage form

In patients who orally administered 30 mg/day 10% 6-MP powder or 50 mg/day AZA tablet (24.0 mg as 6-MP) once a day, the influence of differences in the drug and dosage form on the 6-TGN level was investigated.

2.8. Statistical analysis

The Wilcoxon-signed-ranks test was used for the analysis of steroid dose reduction, and Mann–Whitney's ranks test for the influences of the dosing frequency and drugs with $p<0.05$ being regarded as significant.

3. Results

3.1. Erythrocyte 6-MP metabolite concentrations

The erythrocyte 6-TGN and 6-MMPR levels (mean ± SD) in the 134 patients were 342.3 ± 220.9 and 1283.7 ± 2106.0 pmol/8×10^8 RBC, respectively, showing broad individual variations in the two metabolites levels.

Of 134 patients, the white blood cell count was ≤3000 in 15, AST or ALT was >40 in 5, and the white blood cell count was ≤3000 with AST or ALT>40 in 1, but no patient required a dose change or the discontinuation of 6-MP or AZA. There were no significant differences in the erythrocyte 6-TGN or 6-MMPR level between these patients with abnormal laboratory test values and the other patients.

Table 2 Therapeutic response rates (%) evaluated by activity index

	IBD total (n=134)	UC (n=75)	CD (n=59)	6-MP (n=112)	AZA (n=22)
Remission (n=100)	74.6	65.3 — 84.7 ($P<0.017$)		77.7 — 59.1 (N.S.)	
Non-remission (n=34)	25.4	34.7	15.3	22.3	40.9

N.S.; The difference is not significant.

Table 3 6-TGN levels (pmol/8×10^{8}RBC) in Japanese IBD patients

		UC (n=75)	CD (n=59)	IBD Total (n=134)
Remission	(n=100)	327.5±203.5	373.7±247.4	356.0±231.6
Non-remission	(n=34)	303.3±204.8	361.2±210.0	301.9±183.1
Total	(n=134)	319.1±202.9	371.8±240.5	342.3±220.9

Each data was shown as mean±SD.

3.2. TPMT genotype

No patients with homozygote or heterozygote of TPMT*3C were detected. Neither G460A nor G238C mutation was detected in any subject enrolled in this study. All of the 134 patients were the TPMT wild-type individuals.

3.3. Relationship between erythrocyte 6-MP metabolite concentrations and clinical activity

Clinical remission was achieved in 100 (74.6%) but not in 34 (25.4%) of the 134 IBD patients: 65.3% of the UC patients and 84.7% of the CD patients. The response rate was higher in the CD than in the UC patients. There was no significant difference in the remission rate between 6-MP treatment group and AZA treatment group (Table 2).

The erythrocyte 6-TGN levels (mean±SD) in patients who did and did not achieve remission were 356.0±231.6 and 301.9±183.1 pmol/8×10^8 RBC, respectively. There was no significant difference in the mean erythrocyte 6-TGN levels between the remission and non-remission groups.

In the individual diseases, the erythrocyte 6-TGN level in the 75 UC patients was 319.1±202.9 pmol/8×10^8 RBC: 327.5±203.5 and 303.3±204.8 pmol/8×10^8 RBC in the 49 patients who achieved remission and 26 patients who did not, respectively. The erythrocyte 6-TGN level in the 59 CD patients was 371.8±240.5 pmol/8×10^8 RBC: 373.7±247.4 and 361.2±210.0 pmol/8×10^8 RBC in the 50 patients who achieved remission and 9 patients who did not, respectively. There was no significant difference in the mean erythrocyte 6-TGN levels between the remission and non-remission groups, but the level was lower in the non-remission group in both the UC and CD patients (Table 3).

3.4. Steroid sparing effect

The PSL dose (mean±SD) was 15.4±9.9 mg/day at the time of 6-MP or AZA treatment initiation, and 3.1±5.2 mg/day at the time of 6-MP metabolite measurement (after administration for 3 months or longer). The PSL dose was significantly reduced by 6-MP or AZA treatment ($p<0.01$), and PSL could be withdrawn from 58.4% of the patients (Fig. 1). The erythrocyte 6-TGN level (mean±SD) was 318.1±217.6 pmol/8×10^8 RBC at the time of 6-MP metabolite measurement, attaining the therapeutic target concentration in Europeans/Americans.

3.5. Influence of regimen differences on the erythrocyte 6-TGN concentration

The erythrocyte 6-TGN level (mean±SD) was 397.0±218.4 pmol/8×10^8 RBC in the once-a-day 6-MP (30 mg) or AZA (50 mg) treatment group (71 patients), and 217.2±150.9 pmol/8×10^8 RBC in the 3 times-a-day 6-MP (30 mg)

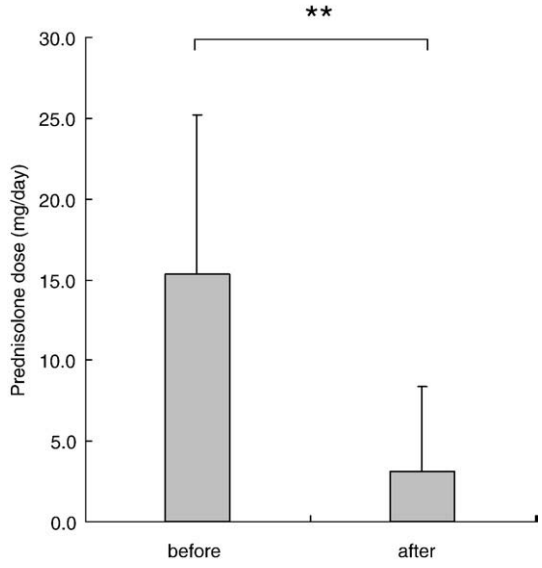

Figure 1 Comparison of prednisolone doses before 6-MP or AZA treatment initiation and those after continual administration for three months or longer (n=76). The prednisolone dose was significantly reduced by 6-MP or AZA treatment (**$p<0.01$) for three months or longer.

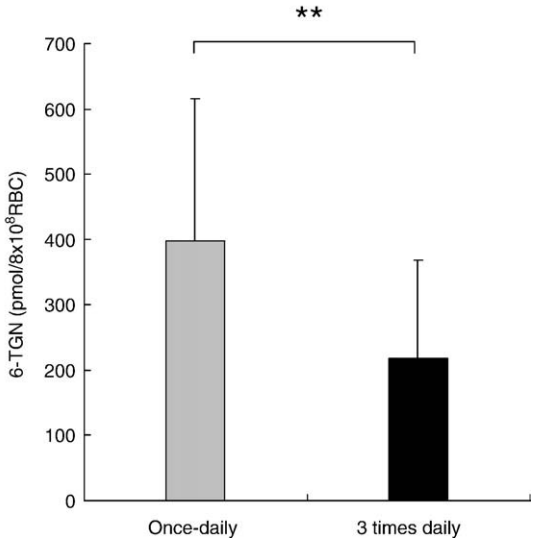

Figure 2 Comparison of erythrocyte 6-TGN concentrations in patients receiving once-a-day 6-MP treatment and those receiving three times-a-day treatment (n=91). The mean 6-TGN level was significantly higher in the once-a-day 6-MP treatment (**$p<0.01$).

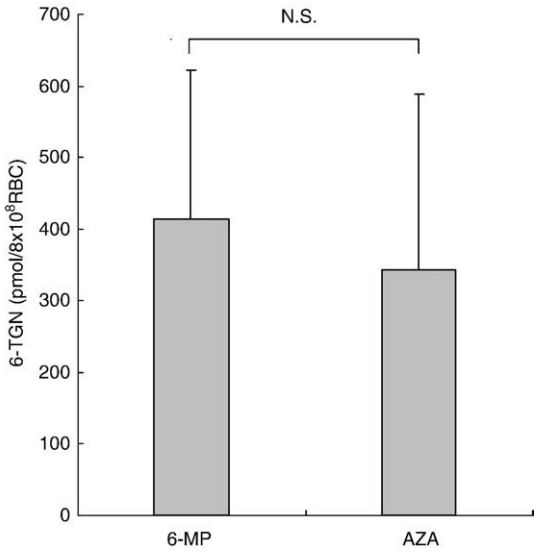

Figure 3 Comparison of erythrocyte 6-TGN concentrations between patients treated with 30 mg of 10% 6-MP powder and patients treated with a 50 mg AZA tablet ($n=71$). 6-MP or AZA was administered once a day. The dose of 50 mg AZA was calculated to the equivalent dose of 24 mg 6-MP. The mean erythrocyte6-TGN concentration was lower in patients receiving the 50 mg AZA tablet, but the difference was not significant (N.S.).

treatment group (20 patients), showing that the level was significantly higher in the once-a-day group ($p<0.01$) (Fig. 2).

The daily dose (mean ± SD) of the 5-ASA preparation concomitantly administered to these patients was 2.1 ± 0.7 g in the once-a-day group and 1.8 ± 0.5 g in the 3 times-a-day group, showing no significant difference.

3.6. Influence of drug type on the erythrocyte 6-TGN concentrations

The erythrocyte 6-TGN level (mean ± SD) was 414.3 ± 208.5 pmol/8×10^8 RBC in 54 patients treated with 30 mg of 10% 6-MP powder once a day, and 342.1 ± 246.0 pmol/8×10^8 RBC in 17 patients treated with 50 mg of AZA tablet once a day.

The mean erythrocyte 6-TGN level was low in the AZA (50 mg tablets) treatment group (24.0 mg as 6-MP), but the difference was not significant (Fig. 3). The daily dose of the 5-ASA preparation concomitantly administered to these patients was 2.0 ± 0.8 g in the 6-MP treatment group and 1.8 ± 0.7 g in the AZA treatment group, showing no significant difference.

4. Discussion

Dubinsky and Cuffari reported that the therapeutic target erythrocyte concentration of 6-TGN for IBD is 235–250 pmol/8×108 RBC or higher, and a 450 pmol/8×108 RBC or higher level increases the risk of bone marrow suppression.[4,5,14] In our study, the dose of 6-MP or AZA (mean ± SD) was 29.8 ± 9.9 mg/day, lower than that in Europe/America, but the 6-TGN level (mean ± SD) was 342.3 ± 220.9 pmol/8[4,5,14]10^8 RBC, attaining the therapeutic target concentration in Europeans/Americans, and clinical remission based on the activity index was achieved in 100 of the 134 patients (74.6%), showing a high response rate. The response rate was higher in the CD (84.7%) than in the UC (65.3%) patients, suggesting that CD is more markedly remitted by 6-MP or AZA. There was no significant difference in the remission rate between 6-MP treatment group (77.7%) and AZA treatment group (59.1%). 6-MP and AZA also contributed PSL dose reduction, and PSL was withdrawn from 58.4% of the patients. These results suggested that the low doses of 6-MP and AZA proposed in Japan based on clinical experience are sufficient for maintaining remission of IBD in Japanese patients, and contribute to the avoidance of severe adverse effects which are a concern of long-term PSL treatment.

Although the mean erythrocyte 5-TGN levels was lower in the non-remission than in remission group in both the UC and CD patients, there was no significant difference between the two groups. The variation of the 6-TGN level was wide, showing large individual variation. In some patients, erythrocyte 5-TGN level reached to 450 pmol/8×108 RBC or higher without any adverse effect requiring discontinuation of the treatment, such as bone marrow suppression. Based on these findings, further data accumulation is necessary to establish the effective target 6-TGN concentration in erythrocytes and the bone marrow suppression-inducing concentration in Japanese IBD patients.

One reason for the sufficient attainment of the therapeutic target 6-TGN level in Japanese IBD patients at about half of the dose established in Europe/America may be a racial difference in the main 6-MP- and AZA-metabolizing enzymes. The gene polymorphism of an important enzyme catalyzing the methylation of 6-MP and AZA, thiopurine S-methyltransferase (TPMT), is present in Caucasians, Africans, African Americans, Latin Americans, Arabs, and Asians, its relation with TPMT enzyme activity distribution has been reported,[15] and G238C, G460A, and A719G have been shown to be the major single nucleotide polymorphism (SNP) involved in the enzyme activity reduction.[16] Lennard reported a patient in whom TPMT activity was reduced, and the 6-TGN level was abnormally high.[17] However, all of the 134 IBD patients in our study had the wild-type TPMT, which does not explain why the 6-TGN level in Japanese treated at a low dose is equivalent to that in Europeans/Americans treated at an approximately 2 times higher dose. There is less information on the TPMT enzyme activity distribution in Japanese, and there has been no report on TPMT activity in Japanese IBD patients. This study suggested that TPMT activity is lower in Japanese than in Europeans/Americans even in individuals with the wild-type TPMT, for which further investigation is necessary.

Some physicians divide 6-MP or AZA administration into 2 or 3 times a day, which may aim at rapid attainment of the steady state of the plasma drug level because the plasma half-lives of the unchanged forms of these drugs are short (1–2 h), or the avoidance of severe bone marrow suppression by reducing the amount of single administration based on clinical experience. However, the absorption from the intestine and subsequent maximum plasma level vary depending on the dose and regimen. The divided administration of 6-MP and AZA may alter the maximum plasma level, and subsequently change the amounts metabolized by xanthine oxidase (XO) in the liver and transferred into

cells, affecting 6-TGN production. This study suggested that the erythrocyte 6-TGN level after drug treatment for 3 months or longer was affected by the dosing frequency: When the daily dose was the same, the 6-TGN level was significantly higher after once-a-day administration than after 3-times-a-day administration, and the mean 6-TGN level in the 3-times-a-day group may not have reached the therapeutic target concentration in Europeans/Americans: 235–250 pmol/8×108 RBC or higher. For the potency and duration of the pharmacological effect of 6-MP and AZA, not only the plasma level but also the cellular level is an important index. The cellular half-life has been reported to be 1–2 weeks,[18] suggesting that once-a-day administration is appropriate for 6-MP and AZA, and contributes to increased patient compliance.

Bioavailability variations of oral 6-MP and AZA are large, and have been reported to be 5–37% of 6-MP[19] and 27–83% of AZA[20]. The selection of 6-MP or AZA for the treatment of IBD is decided by physicians in consideration of each patient's preference for the dosage form. However, there is no reliable information on comparison of the erythrocyte 6-TGN levels between 10% 6-MP powder and AZA film-coating tablet at the same dose by the same regimen. When the daily dose of the concomitant 5-ASA preparation was equivalent, there was no significant difference in the mean erythrocyte 6-TGN level between patients treated with 30 mg of 6-MP (10% powder) and 50 mg of AZA (tablet) (24.0 mg as 6-MP) once a day, clarifying that both dosage forms reached the therapeutic target level established in Europe/America: 235–250 pmol/8×10^8 RBC or higher. Thus, both dosage forms can be selected depending on the wishes and degree of gastrointestinal symptoms of individual patients. However, the inhibition of TPMT activity by the 5-ASA preparation in in-vitro and ex-vivo experiments has been reported,[21] and package inserts of 6-MP and AZA recommend to carefully administer the drug when it is concomitantly administered with a 5-ASA preparation because it may induce severe leukopenia. Concomitant 5-ASA preparations are frequently administered in drug therapy for IBD, and its dose for CD is higher than that for UC. The influence of a dose reduction or escalation of concomitant 5-ASA on the erythrocyte 6-TGN level is unclear. Investigation of the drug interaction between 6-MP/AZA and 5-ASA using the erythrocyte 6-TGN level is necessary.

Since the erythrocyte 6-TGN level is considered to reach a steady state and exhibit a clinical effect after administration for 8–12 weeks,[3,18] patients who had been treated with the drug for 3 months or longer were selected in this study. However, patients who discontinued the treatment due to an adverse effect relatively early after treatment initiation may not have been included because adverse effects of 6-MP/AZA develop relatively early after treatment initiation.[22] For the administration of 6-MP/AZA to IBD patients, treatment should be carefully progressed by closely monitoring clinical findings from immediately after treatment initiation. To design 6-MP/AZA treatment for individual Japanese IBD patients, it is necessary to investigate the background of patients who require discontinuation or dose reduction of 6-MP/AZA due to adverse effects.

This study confirmed that the doses of 6-MP (30 mg/day) and AZA (50 mg/day) applied for the treatment of Japanese IBD patients based on clinical experience, which are about half of the European/American doses per body weight, sufficiently attain the therapeutic target level of 6-TGN, and are safe, improving the QOL of Japanese IBD patients.

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Authors' contribution: TK, TY, RK and TH designed the study and drafted the manuscript. TY, YI, AS and SF collected samples and gave clinical information. KN, IM, YT and HM carried out the sample analysis and performed statistical analysis. All the authors made substantial contributions analysis and interpretation of data.

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