

## Azathioprine formulation optimizes metabolite profile in inflammatory bowel disease

T. STEVENS\*, J. P. ACHKAR\*, K. EASLEY†, A. BRZEZINSKI\* & B. LASHNER\*

\*Department of Gastroenterology and Hepatology, Center for Inflammatory Bowel Disease, Cleveland Clinic Foundation, Cleveland, OH; †Department of Biostatistics, Rollins School of Public Health, Emory University, Atlanta, GA, USA

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### SUMMARY

**Background:** Recent studies have suggested that mercaptopurine metabolism is influenced by drug formulation (mercaptopurine vs. azathioprine) and concomitant use of 5-aminosalicylic acid medications.

**Aim:** To determine the influence of dose, formulation and 5-aminosalicylic acid use on mercaptopurine metabolism.

**Methods:** Metabolites from 131 inflammatory bowel disease patients were analysed. Logistic regression was used to analyse correlations between dose and metabolite levels. Multivariate analysis was used to determine the effects of drug formulation and 5-aminosalicylic acid use.

**Results:** A positive correlation was detected between dose and 6-tioguanine nucleotides levels for azathioprine/Imuran formulation ( $P = 0.005$ ) but not for

mercaptopurine formulation. Adjusted mean 6-tioguanine nucleotides levels were similar for both formulations. Adjusted mean 6-methylmercaptopurine levels were higher for mercaptopurine formulation than for azathioprine formulation (1950 vs. 1056,  $P = 0.04$ ). 5-aminosalicylic acid use: 6-tioguanine nucleotides levels did not differ based on concomitant 5-aminosalicylic acid use. However, 5-aminosalicylic acid use did result in higher adjusted mean 6-methylmercaptopurine levels: 2078 on 5-aminosalicylic acid vs. 991 off 5-aminosalicylic acid ( $P = 0.004$ ).

**Conclusions:** (i) Azathioprine may have metabolic benefits by achieving a correlation of dose with 6-tioguanine nucleotides levels and by leading to lower mean 6-methylmercaptopurine levels. (ii) 5-aminosalicylic acid use does not significantly impact 6-tioguanine levels and may lead to higher 6-methylmercaptopurine levels.

### INTRODUCTION

The purine analogues azathioprine (AZA) and mercaptopurine (6-mercaptopurine) are frequently used to treat patients with inflammatory bowel disease (IBD). AZA is a prodrug that is converted rapidly through a non-enzymatic process to mercaptopurine (Figure 1). Mercaptopurine is subsequently metabolized through three competing pathways: (i) methylation to 6-methylmercaptopurine (6-MMP) metabolites via the enzyme

thiopurine S-methyltransferase (TPMT), (ii) production of the active metabolites 6-tioguanine (6-thioguanine) nucleotides (6-TGN) through the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT), or (iii) conversion to 6-thiouric acid, by xanthine oxidase.<sup>1</sup>

Recent studies have suggested that the measurement of mercaptopurine metabolite levels may be useful in predicting efficacy or guiding therapy with mercaptopurine and AZA in patients with IBD.<sup>2–6</sup> In a recent study, we found a significant correlation of 6-TGN levels with clinical response. Furthermore, there was maximum differentiation between responders and non-responders at a 6-TGN level  $>260 \text{ pmol}/8 \times 10^8 \text{ RBC}$ .<sup>5</sup> Other studies have confirmed these findings,

Correspondence to: Dr T. Stevens, Department of Gastroenterology, Desk A30, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, USA.  
E-mail: stevent@ccf.org

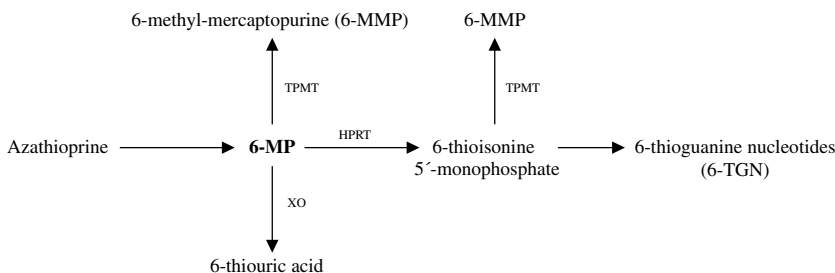


Figure 1. Metabolism of mercaptopurine (6-mercaptopurine) and azathioprine. TPMT, thiopurine S-methyltransferase; HPRT, hypoxanthine-guanine phosphoribosyltransferase; XO, xanthine oxidase.

demonstrating that 6-TGN levels ranging from 230 to 260 pmol/ $8 \times 10^8$  erythrocytes correlate with clinical response, whereas elevated 6-MMP levels are associated with hepatotoxicity. However, other studies have yielded conflicting results.<sup>7, 8</sup>

Several factors such as drug dose, drug formulation, and concomitant use of 5-aminosalicylic acid (5-ASA) medications have been proposed to influence metabolite levels. A meta-analysis of published studies of mercaptopurine/AZA in IBD<sup>9</sup> as well as our own prior study<sup>5</sup> demonstrated a relationship between cumulative dose and clinical response. However, a study by Dubinsky *et al.* failed to show a significant correlation of dose with response, and only a weak correlation of dose with 6-TGN.<sup>3</sup> Although higher doses may be associated with higher 6-TGN levels, a close and reliable correlation has not been supported by the available literature.

Some investigators have proposed that the specific drug formulation may affect metabolism. A recent study by Cuffari *et al.* demonstrated that patients on brand name Imuran and mercaptopurine achieved higher 6-TGN levels than those patients on generic AZA.<sup>10</sup> Moreover, patients on brand name Imuran achieved equivalent 6-TGN levels but required lower doses than patients on mercaptopurine. Based on these results, the authors suggested brand name Imuran was superior to the other formulations.

Finally, factors that affect TPMT, the major enzyme that shunts metabolism of mercaptopurine away from the active 6-TGN metabolites could be clinically relevant in treating patients with IBD. *In vitro* studies have demonstrated inhibition of TPMT by 5-ASA preparations leading to higher 6-TGN and lower 6-MMP levels.<sup>11</sup> A recent clinical study of patients on mercaptopurine or AZA showed an increased rate of leukopenia after the addition of 5-ASA preparations.<sup>12</sup>

Therefore, the aim of this study was to further evaluate the influence of drug dose, drug formulation and the use of concomitant 5-ASA medications on mercaptopurine metabolism in adult patients with IBD.

## MATERIALS AND METHODS

### Study design

Adult patients with IBD on therapy with mercaptopurine or AZA who had metabolite levels measured as part of routine clinical care was studied. Only patients who were on a stable dose of mercaptopurine or AZA for at least 2 months prior to measurement of metabolite levels to ensure equilibration of metabolite levels.<sup>13</sup>

### Data collection

Data collected by review of medical records included age, gender, mercaptopurine or AZA formulations, dose, duration of therapy and concomitant use of 5-ASA medications. The exact drug formulation (i.e. Imuran vs. AZA vs. mercaptopurine) was verified by direct contact at the time of a follow-up visit.

### Statistical methods

In order to allow for direct comparisons between mercaptopurine and AZA, the dose of mercaptopurine (mg/kg) was adjusted by multiplying by a conversion factor of 2.07.<sup>13</sup> Metabolite levels and drug dose were initially summarized with the median and interquartile range for each drug formulation. The Kruskal–Wallis test was used to compare metabolites and dose by drug formulation.

Correlations between 6-TGN or 6-MMP and drug dose were evaluated and compared by ANCOVA. Because the metabolite level data were not normally distributed, a natural log-transformation was performed prior to ANCOVA. A dose-adjusted mean was calculated for each metabolite for each drug formulation subgroup.

The ANCOVA was performed separately for each metabolite to adjust for the use of 5-ASA. Dose adjusted mean values and 95% confidence intervals (CI) for these data are reported based on back transformation of the

log-values to the usual arithmetic scale. The 95% CI were used to summarize the reliability of the sample mean.

## RESULTS

### Demographics

A total of 131 patients with IBD on a stable dose of mercaptopurine or AZA for at least 2 months were evaluated. The mean age of the group was 49 years (range: 15–76), and 58% of the patients were female. About 108 patients (82%) had Crohn's disease, 19 (15%) had ulcerative colitis and four (3%) had indeterminate colitis.

We were able to confirm exact drug formulation for 121 patients (92%). These were the patients that were included in the analysis of drug formulation. Sixty-four patients (53%) were on mercaptopurine, 42 (35%) were on generic AZA and 15 (12%) were on brand name Imuran. Mean total time on therapy was 17 months (range: 2–86); mean time on a stable dose was 9 months (range: 2–70). The median 6-TGN level for the entire group was 269 pmol/ $8 \times 10^8$  erythrocytes (range: 55–761), and the median 6-MMP level was 1620 pmol/ $8 \times 10^8$  erythrocytes (range undetectable to 26 181). Table 1 summarizes the metabolite data and dose by drug formulation.

### Effect of drug dose on mercaptopurine metabolite levels

After adjusting the dose of mercaptopurine (multiplying by factor of 2.07) to allow for direct comparison with AZA and Imuran doses, linear regression was used to examine the relationships between drug dose and metabolite levels. The metabolite data for AZA and

Imuran were similar, so the data for the two agents were combined (AZA/Imuran) for further analysis of drug dose.

A positive linear relationship was detected between dose and 6-TGN levels for AZA/Imuran ( $P = 0.005$ ) but not for mercaptopurine ( $P = 0.64$ ) (Figure 2a). A positive linear relationship was found between dose and 6-MMP levels for both AZA/Imuran ( $P < 0.001$ ) and mercaptopurine ( $P < 0.001$ ) (Figure 2b).

### Choice of drug formulation

The effect of drug formulation on metabolite levels was analysed using multivariate analysis to adjust for drug dose and concomitant 5-ASA use. Mean 6-TGN levels did not differ according to drug formulation (252 for mercaptopurine vs. 241 pmoles/ $8 \times 10^8$  erythrocytes for AZA/Imuran,  $P = 0.68$ ) (Table 2); however, drug formulation did affect 6-MMP levels. Patients on mercaptopurine had higher adjusted mean 6-MMP levels [1950 vs. 1056 pmoles/ $8 \times 10^8$  erythrocytes ( $P = 0.04$ )] than those on AZA/Imuran.

### Use of 5-ASA medications

Sixty-one patients (47%) were on a 5-ASA agent at the time of metabolite measurement. The mean duration of 5-ASA therapy was 27 months (range: 2–180). Of those patients on a 5-ASA drug, 56 (92%) were on mesalazine (mesalamine) (mean dose 3.7 g/day) and five (8%) were on sulfasalazine (mean dose 3.5 g/day).

The effect of 5-ASA medications on metabolite levels was analysed using multivariate analysis to adjust for drug dose and formulation. The concomitant use of 5-ASA did not affect adjusted mean 6-TGN levels (mean 6-TGN = 251 pmoles/ $8 \times 10^8$  erythrocytes for patients on 5-ASA vs. 242 pmoles/ $8 \times 10^8$  erythrocytes for patients off 5-ASA,  $P = 0.68$ ). Adjusted mean 6-MMP levels were significantly higher for patients on 5-ASA than those off 5-ASA (2078 vs. 991 pmoles/ $8 \times 10^8$  erythrocytes,  $P = 0.004$ ) (Table 2).

### Thiopurine S-methyltransferase levels

Thirty-six patients (27%) of patients had TPMT genotype or enzyme activity measured. Of those 36 patients, three (8%) had intermediate TPMT activity; the rest had normal enzyme activity or genotype. Of the three patients with intermediate TPMT activity,

Table 1. Metabolite levels

	n	Dose <sup>1</sup>	6-TGN <sup>2</sup>	6-MMP <sup>2</sup>
6-MP	64	2.3 (1.0) <sup>3</sup>	275 (216)	3740 (6529)
AZA	42	1.6 (0.8)	260 (166)	389 (1530)
Imuran	15	1.7 (0.7)	171 (150)	578 (1881)
P-value <sup>4</sup>		<0.001	0.08	<0.001

<sup>1</sup> Dose (mg/kg) adjusted for comparison with AZA by multiplying by a factor of 2.07.

<sup>2</sup> Units for metabolite levels are pmol/ $8 \times 10^8$  erythrocytes.

<sup>3</sup> Median values (interquartile range) reported for doses and metabolite levels.

<sup>4</sup> Kruskal–Wallis test (non-parametric ANOVA).

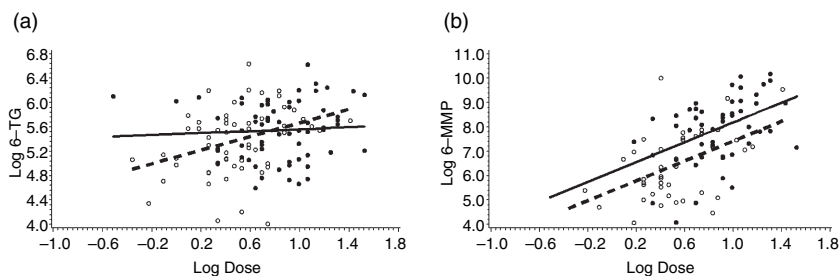


Figure 2. Relationship between dose and mercaptopurine metabolite levels according to drug formulation. (a) Dose vs. 6-thioguanine (6-thioguanine) nucleotides (6-TGN) metabolite levels; (b) dose vs. 6-methylmercaptapurine (6-MMP) metabolite levels. The open circles and dashed lines represent azathioprine. The closed circles and solid lines represent mercaptopurine.

Table 2. Effect of drug formulation and concomitant 5-ASA use on metabolite levels

	Adjusted mean 6-TGN <sup>1,2</sup>	Adjusted mean 6-MMP <sup>1,2</sup>
6-MP	252 (219–289)	1950 (1368–2780)
AZA/Imuran	241 (207–281)	1056 (708–1577)
P-value	0.68	0.04
On 5-ASA	251 (218–289)	2078 (1443–2992)
Not on 5-ASA	242 (211–277)	991 (707–1390)
P-value	0.68	0.004

<sup>1</sup> ( ) represents 95% confidence intervals.

<sup>2</sup> Units for metabolite levels are pmol/8 × 10<sup>8</sup> erythrocytes.

two were taking mercaptopurine and one was taking AZA. The 6-TG levels for these three patients were 751, 287 and 422; the 6-MMP levels were undetectable for two patients and 570 for the remaining patient.

## DISCUSSION

In this study, we evaluated whether certain factors impact on metabolism of mercaptopurine and AZA. We have found a correlation of drug dose with the active metabolite 6-TGN for AZA, but not for mercaptopurine. Furthermore, higher levels of the potentially hepatotoxic metabolite, 6-MMP were seen with mercaptopurine therapy compared with AZA and Imuran. Both of these findings suggest a potential metabolic advantage for the use of AZA formulation. Our results also contradict those of previous studies by finding no difference in 6-TGN levels for those patients taking concomitant 5-ASA medications; in fact, 6-MMP levels were higher in patients on 5-ASA.

A good correlation of dose with 6-TGN levels may allow safer escalation of dose and more predictable efficacy. A study by Dubinsky *et al.* failed to show a significant correlation of mercaptopurine dose with remission, and only a weak correlation of dose with 6-TGN levels.<sup>3</sup>

Although our study detected a statistical correlation of drug dose with 6-TGN levels for AZA, substantial individual variation exists for both formulations, demonstrated by the scatter in Figure 2a. We acknowledge that the clinical significance of this correlation is debatable, and careful monitoring of white blood cell count and metabolite levels remains necessary. The reason that AZA and Imuran exhibit a better correlation of dose with 6-TGN is unclear, but may relate to the low oral bioavailability and variable peak plasma levels of mercaptopurine.<sup>14, 15</sup> Cited reasons for the low bioavailability of mercaptopurine include extensive first pass metabolism by xanthine oxidase,<sup>15</sup> and variable absorption with food.<sup>16</sup> AZA is more easily absorbed than mercaptopurine, perhaps allowing a more predictable increase in blood levels for subsequent conversion to 6-TGN.<sup>17</sup>

The lower 6-MMP levels seen with AZA and Imuran compared with mercaptopurine suggest another potential metabolic advantage for AZA formulation. Higher 6-MMP metabolite have been associated with increased risk for hepatotoxicity.

Although drug equivalency analyses have shown that Imuran and generic AZA have similar oral bioavailability and subsequent metabolism, an investigation by Cuffari *et al.* demonstrated higher 6-TGN levels for brand name Imuran and mercaptopurine compared with generic AZA.<sup>10</sup> Furthermore, patients on Imuran required 44% less drug than those on mercaptopurine to achieve equivalent 6-TGN levels. Our study demonstrated that neither AZA/Imuran nor mercaptopurine formulation favoured the production of the active metabolite, 6-TGN. Furthermore, 6-TGN levels did not differ between generic AZA and Imuran, although there were a low number of patients on Imuran. In fact, there were lower 6-TGN levels for Imuran compared with AZA.

The TPMT enzyme activity strongly influences drug metabolism. The activity of this enzyme is determined by a genetic polymorphism. Patients with low or absent

enzyme activity achieve higher 6-TGN levels and are at greater risk of developing leukopenia.<sup>18, 19</sup> In prior population-based studies 88% of the population were homozygous for the normal TPMT allele (TPMT<sup>H</sup>/TPMT<sup>H</sup>) exhibiting normal TPMT activity, 11% were heterozygous (TPMT<sup>H</sup>/TPMT<sup>L</sup>), exhibiting intermediate activity, and <1% were homozygous for the abnormal allele (TPMT<sup>L</sup>/TPMT<sup>L</sup>), exhibiting low or absent enzyme activity.<sup>20</sup> Although only 27% of patients in our study had TPMT activity assessed, 92% of these had normal activity. The low prevalence of abnormal TPMT activity observed here and in previous studies makes it unlikely that differences in TPMT activity between groups would substantially affect our results.

Drug interactions that alter TPMT activity may affect mercaptopurine metabolite levels. *In vitro* studies have shown an inhibitory effect of 5-ASA compounds on TPMT, which would theoretically lead to higher 6-TGN levels and lower 6-MMP levels.<sup>10</sup> Likewise, a case report documented significant bone marrow suppression with advancement of 5-ASA dose in a patient on mercaptopurine.<sup>21</sup> A potential drug interaction of mercaptopurine or AZA with 5-ASA derivatives is clinically relevant and warrants further investigation, because many IBD patients are on dual therapy (47% of our study group). Some have even suggested the potential for using 5-ASA drugs to manipulate the metabolism of mercaptopurine towards achieving a therapeutic response in resistant patients.<sup>22</sup>

A recent study demonstrated a significant effect of 5-ASA on mercaptopurine metabolism *in vivo*.<sup>12</sup> Thirty-four patients with Crohn's disease who were already taking mercaptopurine or AZA at baseline were enrolled in an 8-week open-label trial of three different 5-ASA formulations. Patients taking mesalazine and sulfasalazine (but not balsalazide) had increased rates of leukopenia (WBC < 3.5) compared with baseline (five of 10 patients in mesalazine group, six of 12 sulfasalazine, two of 12 balsalazide). Similarly, mean whole blood 6-TGN levels increased after addition of 5-ASA, although mean duodenal mucosal 6-TGN levels remained unchanged. Of note, 6-MMP levels were not evaluated in this study.

Our study contradicts these results by showing a lack of effect of concomitant 5-ASA medications on 6-TGN levels. Furthermore, the higher 6-MMP levels observed in patients on 5-ASA suggests a possible potentiation, rather than inhibition of TPMT. One possible explanation for the lack of effect of 5-ASA observed in our study is the order of introduction of

the medications. In the study of Lowry *et al.*, patients were taking mercaptopurine prior to the introduction of 5-ASA.<sup>6</sup> In our study, a majority of the patients were taking 5-ASA before starting mercaptopurine or AZA. One may speculate that the effect of 5-ASA on mercaptopurine metabolism may be most pronounced at the onset of 5-ASA therapy. Although unrecognized confounders may exist in our retrospective analysis, the interaction between 5-ASA and mercaptopurine metabolism is only partly understood and deserves further study.

In summary, we have shown that the prodrug AZA may possess metabolic advantages over mercaptopurine, including a linear correlation of dose with 6-TGN levels, and lower 6-MMP levels. We also have found that 5-ASA use does not significantly impact 6-TGN levels, and may actually lead to higher 6-MMP levels. The clinical significance of these findings deserves further prospective study.

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