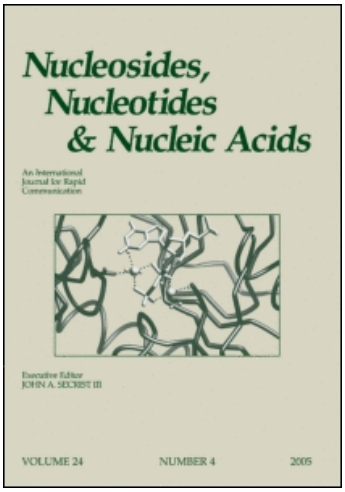

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M. Lindqvist[a]; U. Hindorf[b]; S. Almer[c]; P. Söderkvist[d]; M. Ström[c]; H. Hjortswang[c]; C. Peterson[a]
[a] Department of Medicine and Care, Division of Clinical Pharmacology, Faculty of Health Sciences, Linköping University, Linköping, Sweden [b] Department of Clinical Sciences, Division of Gastroenterology, Faculty of Medicine, Lund University, Lund, Sweden [c] Department of Molecular and Clinical Medicine, Division of Gastroenterology and Hepatology, Faculty of Health Sciences, Linköping University, Linköping, Sweden [d] Department of Biomedicine and Surgery, Division of Cell Biology, Faculty of Health Sciences, Linköping University, Linköping, Sweden

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NO INDUCTION OF THIOPURINE METHYLTRANSFERASE DURING THIOPURINE TREATMENT IN INFLAMMATORY BOWEL DISEASE

M. Lindqvist □ *Department of Medicine and Care, Division of Clinical Pharmacology, Faculty of Health Sciences, Linköping University, Linköping, Sweden*

U. Hindorf □ *Department of Clinical Sciences, Division of Gastroenterology, Faculty of Medicine, Lund University, Lund, Sweden*

S. Almer □ *Department of Molecular and Clinical Medicine, Division of Gastroenterology and Hepatology, Faculty of Health Sciences, Linköping University, Linköping, Sweden*

P. Söderkvist □ *Department of Biomedicine and Surgery, Division of Cell Biology, Faculty of Health Sciences, Linköping University, Linköping, Sweden*

M. Ström and H. Hjortswang □ *Department of Molecular and Clinical Medicine, Division of Gastroenterology and Hepatology, Faculty of Health Sciences, Linköping University, Linköping, Sweden*

C. Peterson □ *Department of Medicine and Care, Division of Clinical Pharmacology, Faculty of Health Sciences, Linköping University, Linköping, Sweden*

□ *The aim of this study was to follow, during standardized initiation of thiopurine treatment, thiopurine methyltransferase (TPMT) gene expression and enzyme activity and thiopurine metabolite concentrations, and to study the role of TPMT and ITPA 94C > A polymorphisms for the development of adverse drug reactions. Sixty patients with ulcerative colitis or Crohn's disease were included in this open and prospective multi-center study. Thiopurine naïve patients were prescribed azathioprine (AZA), patients previously intolerant to AZA received 6-mercaptopurine (6-MP). The patients followed a predetermined dose escalation schedule, reaching target dose at Week 3; 2.5 and 1.25 mg/kg body weight for AZA and 6-MP, respectively. The patients were followed every week during Weeks 1–8 from baseline and then every 4 weeks until 20 weeks. TPMT activity and thiopurine metabolites were determined in erythrocytes, TPMT and ITPA genotypes, and TPMT gene expression were determined in whole blood. One homozygous TPMT-deficient patient was excluded. Five non compliant patients were withdrawn during the first weeks. Twenty-seven patients completed the study per protocol; 27 patients were withdrawn because of adverse events. Sixty-seven percent of the withdrawn patients tolerated thiopurines at a lower dose at Week 20.*

Address correspondence to M. Lindqvist, Department of Medicine and Care, Division of Clinical Pharmacology, Faculty of Health Sciences, Linköping University, SE-58185, Linköping, Sweden. E-mail: malin.lindqvist@imv.liu.se

There was no difference in baseline TPMT enzyme activity between individuals completing the study and those withdrawn for adverse events ($p = 0.45$). A significant decrease in TPMT gene expression (TPMT/huCYC ratio, $p = 0.02$) was found, however TPMT enzyme activity did not change. TPMT heterozygous individuals had a lower probability of remaining in the study on the predetermined dose ($p = 0.039$). The ITPA 94C > A polymorphism was not predictive of adverse events ($p = 0.35$).

Keywords Thiopurine methyltransferase; Enzyme induction; Inflammatory bowel disease; TPMT gene expression

INTRODUCTION

Thiopurines (6-mercaptopurine, 6-MP; azathioprine, AZA; and 6-thioguanine, 6-TG) are widely used in the treatment of inflammatory bowel disease (IBD, ulcerative colitis and Crohn's disease), childhood acute lymphoblastic leukemia (ALL) and after organ transplantation. AZA and 6-MP are used in patients with Crohn's disease and ulcerative colitis to reduce disease activity and the need for corticosteroids, to maintain remission and to prevent relapse. However, up to 40% of patients fail to benefit from the treatment.[1] Individual variations in drug metabolism are of importance for differences in tolerance to thiopurines, which undergo extensive metabolic transformations resulting in the formation of several active and inactive metabolites, including phosphorylation into thioguanosine nucleotides (TGN), and methylation by the polymorphic enzyme thiopurine methyltransferase (TPMT) into methyl-TIMP (meTIMP) and meMP.[2] Drug therapy with thiopurines has been reported to lead to a variable increase or decrease in TPMT enzyme activity, which could alter optimal drug dose and therapeutic outcome.

The aim of this study was to investigate the influence of thiopurine treatment on *TPMT* gene expression and TPMT enzyme activity, and to investigate other pharmacogenetic and pharmacokinetic effects during initiation of thiopurine therapy in patients with inflammatory bowel disease.

MATERIALS AND METHODS

IBD patients with Crohn's disease or ulcerative colitis in whom thiopurine treatment was indicated were included (n = 60). Thiopurine naïve patients were prescribed AZA. Patient previously intolerant to AZA were prescribed 6-MP. The target dose for AZA was 2.5 mg/kg body weight, and for 6-MP, 1.25 mg/kg body weight. The patients followed a predetermined dose escalation schedule and the target dose was reached at week 3. Patients visited the outpatient clinic at baseline (Week 0) and at Weeks 1–8, 12, 16, and 20 after start of treatment. Blood was drawn at each visit for the

isolation of DNA and RNA and for measurements of TPMT, TGN and meTIMP. Real-time RT-PCR for quantification of *TPMT* gene expression was performed as previously described.[3] TPMT enzyme activity and thiopurine nucleotides were assayed in red blood cells as previously described.[4] Genotyping for the *TPMT* and *ITPA* 94C > A polymorphisms was achieved by pyrosequencing.[5–7] Data are presented as median (quartile 1; quartile 3).

RESULTS

One patient was excluded because she lacked TPMT activity and 5 patients were withdrawn during the first 3 weeks of the study because they did not adhere to the dose escalation schedule. Of the 54 remaining patients, 27 patients completed the 20-week period as per protocol (PP-group) and 27 patients were withdrawn during the course of the study because of thiopurine-related adverse events (AE-group). There was a significant decrease in *TPMT* gene expression (*TPMT/huCYC* ratio) during thiopurine treatment ($p = 0.02$, Figure 1A). The median TPMT activity at baseline was comparable in the study groups, for the PP group it was 12.1 (10.7; 13.2) U/ml pRBC and for the AE group, 11.8 (9.4; 14.0) U/ml pRBC ($p = 0.45$). In the PP-group, the TPMT enzyme activity did not differ at subsequent weeks compared to baseline ($p > 0.05$, Figure 1B).

One individual was genotyped as *TPMT*3A/*14*, 7 individuals (12%) were heterozygous for the nucleotide substitutions 460G > A and 719A > G (*TPMT*1/*3A*) and 52 patients (87%) were genotyped as wild type for the *TPMT* gene (*TPMT*1/*1*).

Fifty-three patients (88%) were wild type for *ITPA* 94C > A (C/C), 6 (10%) were heterozygous for *ITPA* 94C > A (C/A) and one patient (1.7%) was homozygous for the variant allele (A/A), giving the allelic frequencies for the C and A alleles of 0.93 and 0.06, respectively. In total, 39 of the patients included in this study experienced adverse events. Twenty-seven

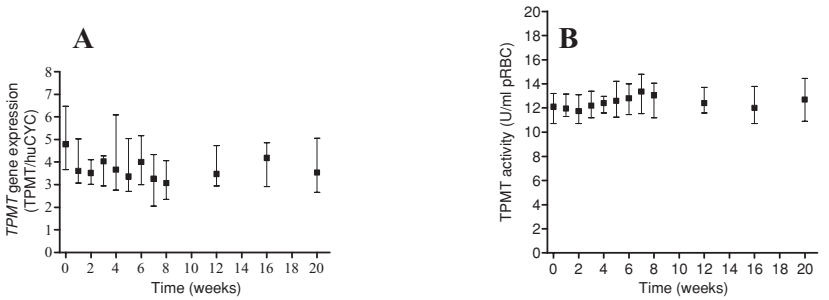

FIGURE 1 *TPMT* gene expression expressed as a ratio between *TPMT* and the gene cyclophilin (*huCYC*, A, n = 16) and red blood cell TPMT enzyme activity (B, n = 27) in the per protocol (PP)-group during the 20-week course of the study. Values are median (quartile 1; quartile 3).

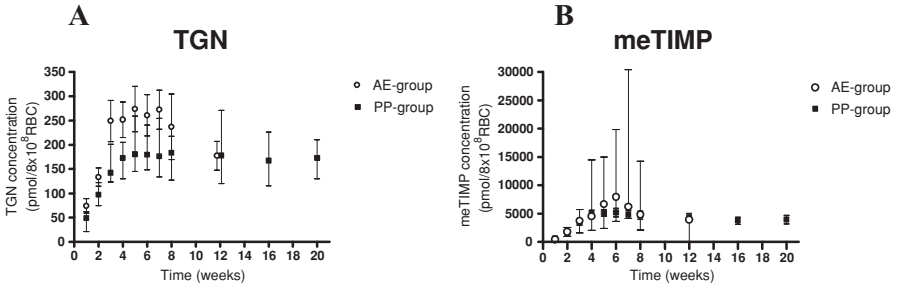

FIGURE 2 Median (quartile 1; quartile 3) of TGN (A) and meTIMP (B) levels during the 20 weeks in the per protocol (PP)-group and adverse events (AE)-group.

patients experienced adverse events which led to discontinued thiopurine therapy (n = 22) or dose reduction (n = 5). Myelotoxicity (n = 9) was the most common cause for withdrawal from the study and led to discontinuation of therapy (n = 6) or a reduction in thiopurine dose (n = 3). Five of six *TPMT* heterozygous patients failed to complete the study due to adverse events, and had a lower probability of remaining in the study compared to *TPMT* wild-type patients (p = 0.039). *TPMT* and ITPA genotype was not significantly predictive of occurrence of adverse events overall (p > 0.35) or subgroups of adverse events (p > 0.05).

Steady state levels of TGN and meTIMP were reached in the PP group after a period of 2 weeks on stable dose, corresponding to week 5 after initiation of treatment (Figures 2A and 2B). Median levels at week 5 for TGN and meTIMP in the PP-group were 180 (145; 259) and 4200 (2600; 7100) pmol/8 $\times$ 10^8 RBC, respectively. Patients in the AE-group formed higher concentrations of TGN and meTIMP (Figures 2A and 2B). However, this difference was statistically significant only for meTIMP at week 6 (p = 0.04). Patients with one variant TPMT allele (*TPMT* 1/* 3A*) formed significantly higher concentrations of TGN during the first 5 weeks of treatment compared to wild type patients.

DISCUSSION

Forty-five percent (n = 27) of patients initially included in this study (n = 60) discontinued thiopurine treatment (n = 22) or required dose reduction (n = 5) because of adverse events. The proportion of patients discontinuing treatment would probably have been lower, if the dose escalation had been less steep. Thus, 13 patients who had discontinued treatment could restart thiopurine treatment at a lower dose within the 20-week period and in total, 67% (18/27) of the withdrawn patients were again or still on thiopurines at week 20.

Interestingly, we found a significant decrease in *TPMT* gene expression during thiopurine treatment. One explanation for this decrease is the

inhibition of purine *de novo* synthesis by meTIMP formed from the thiopurines, leading to a decreased amount of purines available for DNA and RNA synthesis.[8]

In general in our study population, TPMT activity did not change during the treatment with thiopurines, but we observed marked inter-individual differences in parallel to an earlier study.[9] Drug treatment other than the thiopurines and red blood cell age and transfusions are important factors influencing TPMT enzyme activity.[10] In accordance with a previous study,[11] we did not find any association between the *ITPA* 94C > A polymorphism and the emergence of adverse events during thiopurine treatment.

In conclusion, this study presents a detailed description of the influence of thiopurine treatment on *TPMT* gene expression and enzyme activity. *TPMT* gene expression decreased during the thiopurine treatment, whereas TPMT enzyme activity generally did not change. We conclude that TPMT is not induced by thiopurines in inflammatory bowel disease.

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