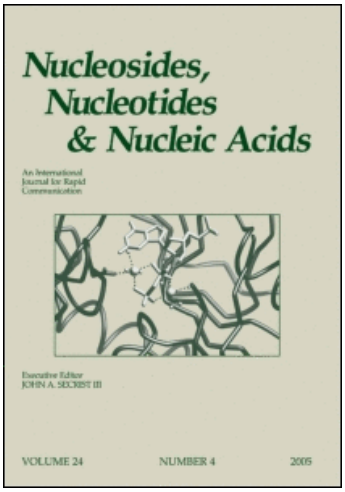

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Limited Intra-Individual Variability in Hypoxanthine-Guanine Phosphoribosyl Transferase, Thiopurine S-Methyl Transferase, and Xanthine Oxidase Activity in Inflammatory Bowel Disease Patients During 6-Thioguanine Therapy

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LIMITED INTRA-INDIVIDUAL VARIABILITY IN HYPOXANTHINE-GUANINE PHOSPHORIBOSYL TRANSFERASE, THIOPURINE S-METHYL TRANSFERASE, AND XANTHINE OXIDASE ACTIVITY IN INFLAMMATORY BOWEL DISEASE PATIENTS DURING 6-THIOGUANINE THERAPY

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□ *6-Thioguanine (6-TG) may be indicated in case of intolerance of or resistance to conventional thiopurines in the treatment of inflammatory bowel diseases (IBD). The aim of our study was to evaluate the intrapatient variability in the 6-TG metabolizing enzymes: hypoxanthine-guanine phosphoribosyl transferase (HGPRT), thiopurine S-methyl transferase and xanthine oxidase. We performed a pharmacokinetic study of 6-TG after oral and intravenous administration in IBD patients in remission. The enzyme activities were determined at baseline and 1 week after the initiation of 6-TG in red blood cells, peripheral blood mononuclear cells (PBMC) or plasma. From the results we conclude that HGPRT activity in erythrocytes decreases following the initiation of 6-TG therapy, which may imply that HGPRT is a rate limiting enzyme in 6-TG metabolism. Moreover, little intrapatient variability in enzyme activities was observed except for HGPRT activity in PBMC. These data may have implications in regard of future therapeutic drug monitoring.*

KEYWORDS Hypoxanthine-guanine phosphoribosyl transferase; thiopurine S-methyl transferase; xanthine oxidase; inflammatory bowel diseases; 6-thioguanine

INTRODUCTION

In the treatment of inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis, thiopurines such as 6-mercaptopurine (6-MP) and its pro-drug azathioprine (AZA) have effectively been used for decades to maintain remission and diminish the use of corticosteroids.[1,2]

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These hypoxanthine analogues are metabolized into pharmacologically active 6-thioguanine nucleotides (6-TGN) in the purine salvage pathway, which involves multiple anabolic and catabolic steps. In addition to 6-TGN, potentially toxic 6-methylmercaptopurine ribonucleotides (6-MMPR) are formed; the amount of which depends for the greater part on thiopurine S-methyl transferase (TPMT) activity. Unfortunately, therapeutic failure frequently occurs and may, at least in part, be due to the formation of these toxic metabolites.

In case of intolerance of or resistance to AZA or 6-MP, 6-thioguanine (6-TG) may be used as an alternative thiopurine. The metabolization of 6-TG, as compared with 6-MP, is far less complex since hypoxanthine-guanine phosphoribosyl transferase (HGPRT) directly converts 6-TG into 6-TGN without the formation of toxic 6-MMPR (Figure 1). Yet, 6-TG is a substrate for TPMT, yielding 6-methyl thioguanine. Other enzymes involved in 6-TG metabolism besides HGPRT and TPMT are guanine deaminase, aldehyde oxidase, and indirectly, xanthine oxidase (XO). Contrary to its relatively short salvage pathway, there is a wide interpatient variability in red blood cell (RBC) 6-TGN concentrations upon 6-TG administration, resulting from different individually determined enzyme activities.[3] Deviant enzyme activities (phenotype) are mainly due to single nucleotide polymorphism in the genes encoding these enzymes. However, altered gene expression or post-transcriptional enzyme modifications may also influence enzyme activities. Accordingly, not only interpatient, but also intrapatient variability in 6-TGN concentrations may occur upon 6-TG administration.

Therefore, the present study was carried out to assess the temporal variability in phenotypes of important enzymes that metabolize 6-TG in a population of IBD patients.

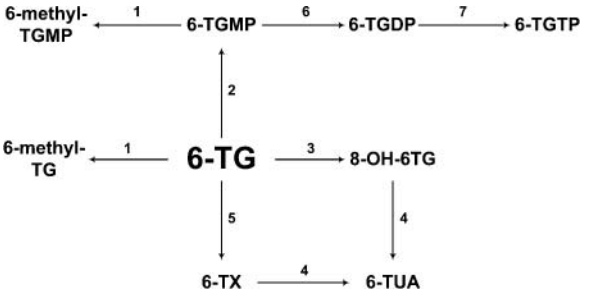

FIGURE 1 Proposed 6-thioguanine (6-TG) metabolism. (1) Thiopurine S-methyl transferase (TPMT); (2) hypoxanthine-guanine phosphoribosyl transferase (HGPRT); 6-thioguanine monophosphate (6-TGMP); (3) aldehyde oxidase (AO); 8-hydroxy-6-thioguanine (8-OH-6TG); (4) xanthine oxidase (XO); 6-thiouric acid (6-TUA); (5) guanine deaminase (GD); 6-thioxanthosine (6-TX); (6) Nucleoside mono- and (7) diphosphate kinases (NMPK and NDPK); 6-thioguanine diphosphate (6-TGDP); 6-thioguanine triphosphate (6-TGTP).

MATERIALS AND METHODS

Patients

Adult IBD patients who had proven to be intolerant of or resistant to AZA or 6-MP and in need of immunosuppressive therapy were eligible for inclusion. In addition, conventional thiopurine therapy had to be withdrawn for at least 4 weeks. Exclusion criteria were: known duodenal or jejunal inflammation due to Crohn's disease, short bowel, active infection, (expected) pregnancy, use of allopurinol, or ACE-inhibitors or furosemide, leukopenia, thrombopenia, anemia, impaired renal, or liver function, and a history of tuberculosis or HIV or hepatitis B or C.

Study Design

We performed an open-label clinical trial with cross-over design that evaluated pharmacokinetic properties of both orally and intravenously administered 6-TG in IBD patients. In these patients enzymatic activities were assessed at baseline and 1 week following initiation of 6-TG therapy. Patients received a single oral (PO) dose of approximately 0.3 mg/kg 6-TG at day 1 followed by a second single dose of 0.3 mg/kg 6-TG intravenously (IV) at day 8, or vice versa. At day 1 demographic and disease specific data were collected and TPMT genotype was determined. Enzyme activities of TPMT, HGPRT, and XO were determined in samples, which were collected on both days just prior to the administration of 6-TG. Red blood cell TPMT, and HGPRT activities were additionally determined in an age and sex matched population. Intrapatient variability in enzyme activities was illustrated by means of analysis of differences between the individual successive measurements. The absolute difference between the successive activities was divided by the baseline activity and expressed as a percentage. The median and range of this percentage was used as a measure of proportional intrapatient variability.

The protocol was approved by the medical ethics review committee of the VU University Medical Center.

Determination of TPMT Genotype

TPMT genotyping of leukocyte DNA was accomplished by means of Restriction Fragment-Length Polymorphism (RFLP) analysis adapted from Yates et al.[4] Patients were screened for presence of the TPMT*2 allele (characterized by the G238C mutation) and the TPMT*3 allele (with subtype A containing the mutations: G460A and A719G, subtype B containing only the G460A mutation and subtype C containing only the A719G mutation).

Enzyme Activities of HGPRT, TPMT, and XO

Enzyme activities were measured at 37°C in a shaking water bath. Enzyme activities of HGPRT in peripheral blood mononuclear cells (PBMC) and RBC were expressed in nmols of product per hour per mg protein. The activity of TPMT in RBC was expressed in pmols per hour per mg protein. Plasma XO activity was expressed in mU per hour per ml, where 1 unit is defined as the amount of XO that will form 1 μmol of uric acid from hypoxanthine. Red blood cells and PBMC were isolated as previously described.[5]

For HGPRT analysis, the RBC pellet was suspended in deionized water, sonicated and diluted in 250 mM Tris-HCI (pH 7.4) containing 25 mM $MgCI_2$. Peripheral blood mononuclear cells were directly suspended in 100 mM Tris-HCl (pH 7.4) containing 25 mM $MgCI_2$ and 1 mM EDTA and sonicated. The HGPRT activity was measured in 20,000 g supernatants diluted with Tris buffers. The incubation mixture (40 μl) for the HGPRT assay for RBC contained 125 mM Tris-HCI (pH 7.4), 12.5 mM $MgCI_2$, 1.86 mM 5-phosporibosyl-1-pyrophosphate (PRPP) (Sigma-Aldrich Corp., St. Louis, MO, USA), RBC supernatant (18–28 μg protein per assay) and 0.15 mM [8-^{14}C] hypoxanthine (47 mCi/mmol, Moravek Biochemicals Inc., Brea, CA, USA). After 15–30 minutes the HGPRT assay for RBC was stopped by heating at 95°C for 5 minutes. The HGPRT assay for PBMC contained 50 mM Tris-HCl (pH 7.4), 12.5 mM $MgCI_2$, 1 mM EDTA, 1.86 mM PRPP and PBMC extracts (equivalent to 2×10^5 cells). The assay for PBMC was stopped by adding 10 μl 8 N formic acid, because of the higher heat stability of the PBMC HGPRT. Separation of nucleotides from nucleosides and bases on polyethyleneimine (PEI)-cellulose and determination of radioactivity was performed as previously described.[6] Thiopurine S-methyl transferase activity in RBC was determined by high-performance liquid chromatography (HPLC) as previously described by Keizer-Garritsen et al.[7] Plasma XO activity was assessed by using the Amplex Red Xanthine/Xanthine Oxidase Assay kit (Invitrogen, Carlsbad, CA, USA).

RESULTS

A total of 18 patients with a mean age of 44.6 (±11.0) years were included in this study. Three patients (17%) were male. Six patients had CD and 12 UC. Twelve patients received 6-TG first PO and then IV, while the remaining 6 received it inversely. TPMT genotype assessment revealed wild-type alleles in all patients. At baseline, Crohn's disease patients had higher HGPRT activities in PBMC as compared with UC patients (331 ± 68 vs. 240 ± 60 nmol/(h × mg protein) ($P = 0.012$) and males had higher TPMT activity as compared with females (32.5 ± 3.6 vs. 23.6 ± 4.5 pmol/(h × mg protein); $P = 0.024$). Healthy controls (n = 18) possessed higher RBC TPMT activity but lower RBC HGPRT activity as compared with IBD patients. Figure 2

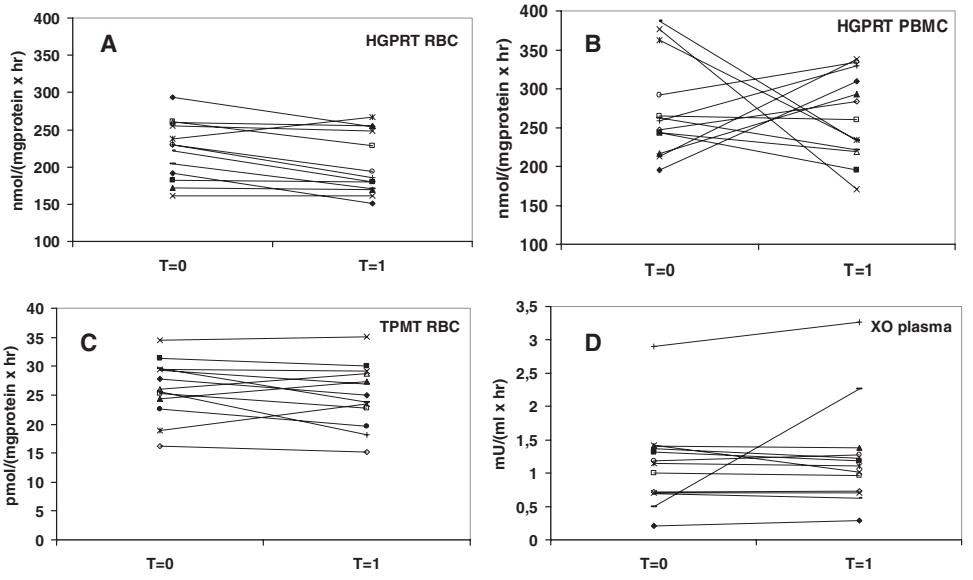

FIGURE 2 Intrapatient variability in enzyme activities. The proportional intrapatient variability was 11% (1–29%) for HGPRT in RBC, 27% (2–59%) for HGPRT in PBMC, 10% (0–21%) for TPMT in RBC and 9% (0–361%) for XO in plasma.

illustrates the enzyme activities that were assessed at baseline and 1 week later. Mean HGPRT activity measured in RBC decreased from 223 (±39) to 203 (±41) nmol/(h × mg protein) ($P = 0.009$). The mean activities of HGPRT in PBMC, TPMT in RBC and XO in plasma, did not statistically significantly change (Table 1). The proportional intrapatient variability was 11% (1–29%), 10% (0–21%), and 9% (0–361%) for HGPRT in RBC, TPMT in RBC and XO in plasma, respectively. For HGPRT in PBMC this proportional intrapatient variability was 27% (2–59%).

TABLE 1 Activities of enzymes involved in 6-thioguanine metabolism are expressed as mean ± S.D.

	Controls	T = 0	T = 1	P (T0 vs. T1)
HGPRT RBC (nmol/(h × mg protein)	156 (24)	223 (39)	203 (41)	.009
HGPRT PBMC (nmol/(h × mg protein))	98 (22)*	274 (63)	263 (56)	.711
TPMT (pmol/(h × mg protein))	30.2 (7.0)	26.2 (5.0)	25.0 (5.4)	.217
XO (mU/(h × ml))	n.a.	1.12 (0.66)	1.23 (0.77)	.456

The control population consisted of healthy adult volunteers. Differences between groups (T = 0 vs. T = 1) were tested using the paired Student's *t* test. Hypoxanthine-guanine phosphoribosyl transferase (HGPRT); red blood cells (RBC); peripheral blood mononuclear cells (PBMC); thiopurine S-methyl transferase (TPMT); xanthine oxidase (XO); not available (n.a.).

*Control values for HGPRT in PBMC were partly adapted from Peters and Veerkamp.[5]

DISCUSSION

The present study assessed the temporal stability of phenotypes of multiple important enzymes in 6-TG metabolism in IBD patients. Except for HGPRT in PBMC, the intrapatient variability in the activities of important enzymes in 6-TG metabolism was fairly low.

In our study population, we observed a mean TPMT activity comparable to that observed in other IBD populations.[8,9] Red blood cell TPMT activity in healthy controls was in the same range of that reported earlier with a similar essay,[7] and higher than in our IBD population. In accordance with a previously published study, TPMT activity was higher in males as compared with females.[10] Importantly, there was little intrapatient variability of TPMT activity and the mean activity did not change over time. A prospective trial evaluating TPMT activity during 1 year of treatment with AZA did neither observe an alteration in TPMT activity,[8] although a higher TPMT activity has previously been related with thiopurine therapy.[9,11]

The mean RBC HGPRT activity in our population was clearly higher than the mean activity in the healthy controls. We assume that the previous long-term use of thiopurines may have induced HGPRT activity. Intriguingly, in our study the mean HGPRT activity in RBC decreased after 6-TG initiation. It might be possible that 6-TG therapy down-regulated the synthesis of HGPRT or decreased its stability. Alternatively, the RBC population might have changed. These results may imply that HGPRT is a rate limiting enzyme in 6-TG metabolism. Contrary to the overall decrease in HGPRT activity in RBC, intrapatient variability was relatively low.

In PBMC, HGPRT activity was slightly higher than in RBC. In addition, CD patients had a higher HGPRT activity in PBMC as compared with UC patients. Although we did not obtain disease activity parameters, we assume that this enhanced activity may be explained by a higher leukocyte activity in CD patients. Purine salvage activity increases in leukocytes after mitogenic stimulation and is accompanied by an enhanced cellular HGPRT activity next to enhanced other salvage enzymes.[12,13]. Intrapatient variability in HGPRT activity if determined in PBMC appeared to be very high, whereas no change in mean activity was observed. This high variation may be due to the intermitting inflammatory status of the patients. In addition, differences in leukocyte subpopulations may arise as a result of changing inflammatory status and consequently the overall PBMC HGPRT activity may alter. The higher PBMC HGPRT activity in IBD patients as compared with historical controls[5] might indicate such an enhanced activation status due to the continuous inflammatory condition of these patients.

Intrapatient variability of XO activity in plasma was comparable to that of HGPRT in RBC and TPMT in RBC. In this study population no difference in mean XO activity between the two longitudinal measurements was observed. These results are in agreement with two other studies that also observed little intrapatient variability in XO activity.[14,15]

In conclusion, this study shows that intrapatient variability in the activities of HGPRT in RBC, TPMT in RBC and XO in plasma are fairly stable except for HGPRT activity in PBMC. In addition, we found HGPRT to be a possible rate limiting enzyme in 6-TG metabolism. These data may have implications in regard of future therapeutic drug monitoring.

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