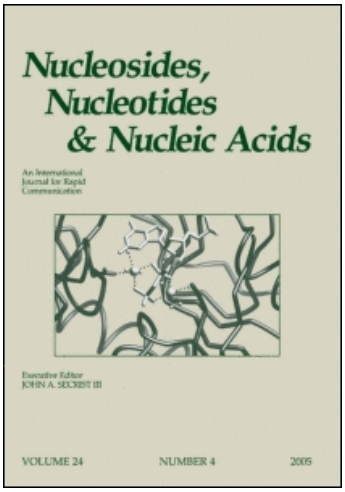

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 27 October 2004

To cite this Article Coulthard, S. A. , Matheson, E. C. , Hall, A. G. and Hogarth, L. A.(2004) 'The Clinical Impact of Thiopurine Methyltransferase Polymorphisms on Thiopurine Treatment', Nucleosides, Nucleotides and Nucleic Acids, 23: 8, 1385 — 1391
To link to this Article: DOI: 10.1081/NCN-200027637
URL: http://dx.doi.org/10.1081/NCN-200027637

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 8 & 9, pp. 1385–1391, 2004

The Clinical Impact of Thiopurine Methyltransferase Polymorphisms on Thiopurine Treatment

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ABSTRACT

Acute lymphoblastic leukaemia (ALL) is the most common malignancy of childhood. Although current treatment results in long term survival in over 70% of cases there is evidence that as many as 50% could have been cured using a less complex regimen with a lower incidence of long term side effects. In previous studies it has been found that thiopurines given as part of continuing therapy are key agents in preventing relapse. However, optimal administration during continuing therapy is often not achieved. Variation in the level of thiopurine methyltransferase (TPMT) activity appears to be a major molecular determinant of the extent of thiopurine metabolism. TPMT activity shows a trimodal distribution pattern. A lack of activity is found in approximately one in 300 Caucasians; approximately 11% have intermediate activity and the remaining 89% high activity. Congenital loss of activity is associated with grossly elevated levels of active drug and profound myelosuppression on exposure to thiopurines. This loss of activity has been attributed to single nucleotide polymorphisms (SNPs) within the TPMT gene. The frequency of SNPs is related to ethnicity, with the most common in Caucasians being TPMT*3A which is characterized by a G to A transition at position 460 with a substitution of alanine for tyrosine at amino acid 154 (A154Y) and a transition of A to G at nucleotide 719 resulting in a change of tyrosine to cysteine at position 240 (Y240C). Polymorphisms

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DOI: 10.1081/NCN-200027637
1525-7770 (Print); 1532-2335 (Online)

have also been identified within the 5′ flanking promoter region of the TPMT gene due to a variable number of tandem repeats (VNTR*3–*8). An overview of the polymorphisms identified to date, their implication on the metabolism of the thiopurine drugs and therapeutic importance will be discussed.

Key Words: Thiopurine methyltransferase (TPMT); 6-mercaptopurine; 6-thioguanine; Azathioprine; Leukaemia.

INTRODUCTION

The thiopurines consist of three drugs: 6-mercaptopurine (6-MP), 6-thioguanine (6-TG) and azathioprine. 6-TG is used in the treatment of childhood Acute Lymphoblastic Leukaemia (ALL) during induction therapy. 6-MP is used during the continuing phase of therapy for ALL. Azathioprine, which is rapidly converted to 6-MP, is a widely used immunosuppressant given to patients with autoimmune conditions, inflammatory bowel disease (Crohn's) and following kidney or heart transplantation. All were designed as purine analogues in order to disrupt normal DNA synthesis and as such are metabolised by the same pathways that control normal nucleotide homeostasis.

In the UK there are approximately 420 new cases of childhood ALL per year of which about 95% will achieve complete remission. Seventy-five percent of these will be cured. Treatment of childhood ALL includes broadly; remission induction (using a glucocorticoid, vincristine and L-asparaginase); intensification therapy (duanorubicin, cytosine arabinodes, etoposide, vincristine prednisolone and 6-TG) and continuing

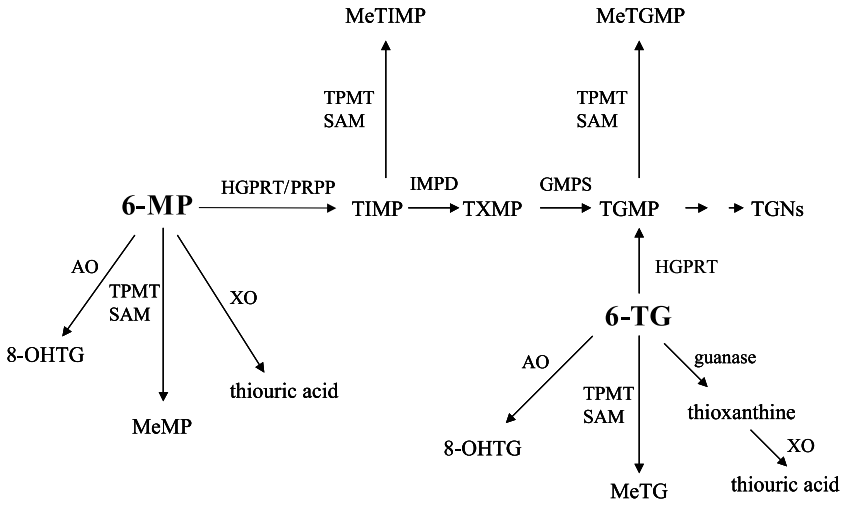

Figure 1. Metabolism of 6-MP and 6-TG in human ALL cells. PRPP, 5′-phosphoribosyl-1-pyrophosphate; GMPS guanosine monophosphate synthase; HGPRT, hypoxanthine guanine phosphoribosyltransferase; IMPD, inosine monophosphate dehydrogenase; SAM, S-adenosine-L-methionine; TPMT, thiopurine methyltransferase; AO, aldehyde oxidase; 8-OHTG, 8-hydroxythioguanine; XO, xanthine oxidase; TGN, thioguanine nucleotides; TIMP, thioinosine 5′-monophosphate; TXMP, thioxanthine monophosphate; TGMP, thioguanosine monophosphate; MeTG methylthioguanine; MeMP, methylmercaptopurine.

therapy (methotrexate and 6-MP). In ALL97/99 6-TG was randomised against 6-MP, however, due to toxicity 6-TG was taken out of the randomisation in April 2002.

Both 6-MP and 6-TG (Fig. 1) are pro-drugs that require activation, by hypoxanthine-guanine phosphoribosyl transferase (HGPRT), and extensive metabolism in order to exert a cytotoxic effect by the incorporation of fraudulent bases, thioguanine nucleotides (TGNs), into the DNA and RNA.[1] Competing with HGPRT, for the metabolism of thiopurines, are three enzymes, thiopurine methyltransferase (TPMT) (EC 2.1.1.67), aldehyde oxidase and xanthine oxidase. In the case of 6-TG, xanthine oxidase can only metabolise the drug after prior conversion by guanase. The products of these competing reactions produce metabolites with little or no cytotoxic activity. Of these three competing pathways, TPMT shows the most variation in activity between individuals. In 1980 Weinshilboum first reported the trimodal distribution of TPMT activity which was measured in the red blood cells (RBC) of 298 individuals. Of these 88.6%[2] had high enzyme activity, 11% intermediate activity, and 0.3% undetectable activity. This distribution pattern has also been shown to occur in a cohort of normal adults and children from the North-East of England by Coulthard et al.[3]

The TPMT gene has been localised to chromosome 6p22.3[4] Variation in activity is now known to be the result of single nucleotide polymorphisms (SNPs), some of which result in reduced enzyme activity in red blood cells[4] and leukaemic blasts.[5] In Caucasian populations, the most common polymorphism associated with reduced enzyme activity, designated *3A, involves point mutations at positions 460 (G□A) and 719 (A□G) whilst an isolated mutation at position 719 (*3C) is the most common cause of low TPMT activity in African populations.[6]

MEASUREMENT OF TPMT

TPMT phenotype and genotype can be measured by several different methods. Phenotype analysis can be measured using a radiochemical activity assay[7,8] or an HPLC-based activity assay. For genotype analysis methods that have been described include restriction fragment[9] length polymorphism,[5,10] denaturing HPLC[11,12] and Pyrosequencing™. Details of the latter can be found at http://www.pyrosequencing.com and details of the Pyrosequencing™ method for TPMT genotyping are currently part of a manuscript in preparation within our laboratory.

IN VITRO MODEL TO DETERMINE THE AFFECT OF TPMT ACTIVITY ON 6-MP AND 6-TG EFFICACY

In order to ascertain the impact of TPMT status on the sensitivity to and metabolism of 6-TG and 6-MP an in vitro model was generated by the transfection of TPMT cDNA, using an inducible vector system, into an embryonic kidney cell line EcR293.[13] Induction of TPMT caused a four-fold increase in TPMT activity, comparable to the range of activity we previously reported in leukaemic blasts.[5] The effect of enhanced TPMT expression on drug sensitivity, MeTIMP production, DNA-TGN incorporation and PDNS was found to differ markedly for 6-MP and 6-TG.[5] Induction of TPMT led to a 1.6-fold increase in resistance when the cells were treated

with 6-TG and, conversely, a 4.4-fold increase in sensitivity when treated with 6-MP. With both drugs, the amount of TGN incorporation was inversely proportional to the TPMT activity as expected. However, for cells treated with equitoxic doses of 6-TG, DNA-TGN levels were equivalent. Exposure of cells to equitoxic doses of drug showed similar incorporation of DNA-TGN for 6-TG but for 6-MP significantly less DNA-TGN in TPMT induced compared with un-induced cells. Induction of TPMT led to an increased production of methyl thioinosine monophosphate (MeTIMP), a potent inhibitor of purine de novo synthesis (PDNS) and increased inhibition of PDNS with 6-MP. Conversely, decrease in PDNS inhibition was observed in cells induced for TPMT with 6-TG.

Following 6-TG treatment there were significantly lower S-adenosyl methionine (SAM) levels in the low TPMT expressing cells but no significant difference in the S-adenosyl homocysteine (SAH) levels. With 6-MP there was no significant difference in SAM levels but a substantial increase in SAH with TPMT induction. Such an increase in SAH levels was not observed on exposure of cells to methylated mercaptopurine riboside, which is converted to MeTIMP within cells. This suggests that the increase in SAH on exposure to 6-MP is not due to increase in MeTIMP in high TPMT expressing cells. SAM is a methyl-donor for DNA methyltransferases (DNA-MTs) and SAH has been shown to inhibit DNA-MTs. Hence, alterations in SAM and SAH levels may cause changes in DNA methylation.[14] This is currently under investigation within our laboratory.

CLINICAL EVIDENCE OF TPMT ACTIVITY ON THIOPURINE DRUG ACTION

RBC TPMT activity has been shown to be inversely related to RBC-TGN concentration in children with leukaemia.[15,16] Blast cell TPMT activity has also recently been shown to correlate significantly with RBC activity at diagnosis.[8] High RBC concentrations of TGNs correlate with the degree of leukopenia and a good prognosis,[17,18] while low concentrations appear to be associated with a higher risk of relapse.[21–24] In patients who are treated with standard doses of thiopurine drugs, those with TPMT deficiency are susceptible to severe myelosuppression when treated with azathioprine[17,19,20] and haematopoietic toxicity when treated with 6-MP.[23,25–27] Even those who have a heterozygous phenotype have been known to suffer severe hepatic toxicity and other adverse side effects.[28] In cases where TPMT deficiency has been identified, 6-MP drug doses have been attenuated with respect to TPMT status.[26,27,29,30]

One important conclusion from our results is that, in cells with high levels of TPMT, 6-MP can exert a cytotoxic effect without measurable levels of TGN incorporated into DNA and, therefore, possibly a reduced risk of mutagenesis posed by TGN incorporation. In cells with low TPMT, however, levels of TGN incorporation in the DNA are much higher for a given level of cytoxicity. These results may provide a mechanistic explanation for the recent observation that the rates of secondary malignancy are raised in patients with congenitally reduced TPMT levels treated with 6-MP.[31,34] The clinical toxicity seen in patients treated with 6-TG has caused the drug to be withdrawn in UKALL97-99.

It is thus clear that variation in TPMT activity has important clinical significance, both for the selection of which agents to use in clinical practice and possibly in the propensity of the drugs to generate DNA mutations.[31–34]

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