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Novel assay to improve therapeutic drug monitoring of thiopurines in inflammatory bowel disease

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Abstract

Background and aims: The thiopurines are widely used in the treatment of inflammatory bowel disease, but are limited by poor dose–effect relationship. The objective was to assess the ability of a novel assay, determining the mono-, di-, and triphosphates, of thioguanine as well as methylthioinosine as individual metabolites in erythrocytes, to predict clinical outcome compared to a routine assay, determining metabolites as sums.
Methods: Samples from 79 patients with Crohn's disease or ulcerative colitis treated with azathioprine or mercaptopurine were analysed by both assays. Clinical status was determined by the Harvey–Bradshaw and Walmsley indices. The genotypes of thiopurine methyltransferase (TPMT) and inosine triphosphatase were determined.
Results: TPMT wild-type patients with thioguanine nucleotide (TGN) levels below the cut-off level were more likely to have active disease when TGN was measured by the novel assay ($p = 0.02$), and when thioguanosine triphosphate (TGTP) was measured separately ($p = 0.01$). When TGN was measured by the routine assay the correlation was not evident ($p = 0.12$). Neither TGN levels nor TGTP correlated to disease activity in TPMT deficient patients. Patients with methyl thioinosine

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nucleotide (meTIN) levels above 1500 pmol/8 × 10^8 RBCs were more likely to have active disease (p = 0.07). We observed good correlations between the mono-, di-, and triphosphates and their respective sums ($R^2 > 0.88$).
Conclusions: The novel TGN assay was better in predicting clinical outcome compared to the routine assay, while determination of TGTP had no clinical advantage and TGTP ratio was not correlated to disease activity.

1. Introduction

Thiopurines constitute the mainstay of immunosuppression in inflammatory bowel disease (IBD). There are three different thiopurines in clinical use today, azathioprine (AZA), mercaptopurine (6-MP), and thioguanine (6-TG). They are pro-drugs believed to be active through the formation of thioguanine nucleotides (TGN) and methylthioinosine nucleotides (meTIN).[1] The metabolism of the different thiopurines can be seen in Fig. 1. The complexity of thiopurine metabolism is illustrated by the fact that it is common that patients intolerant to one thiopurine can tolerate another.[2] The pharmacological explanation for this is not yet completely understood.

The pharmacokinetics of thiopurines not only is complex, but also shows extensive inter-individual variability. One cause of variability is the polymorphism of thiopurine methyltransferase (TPMT).[3] However, even when taking TPMT activity into consideration, inter-patient variability is high. In theory, concentration measurements of active metabolites could be used to tackle this variability but in reality a definite place for therapeutic drug monitoring (TDM) of thiopurines has been difficult to establish, possibly for methodological reasons.

Two assays are commonly used in the TDM of thiopurines,[4,5] both measuring the hydrolysis products of TGN and meTIN in red blood cells (RBCs). In these assays, the nucleotides are hydrolysed back to nucleic bases and then analysed by liquid chromatography. These assays cannot distinguish between nucleotides, ribosides, and their deoxy analogues,[6] and co-determine these as sums. Thus, information about the metabolite distribution is lost. These assays are used for practical reasons, as nucleic bases are easier to analyse than nucleotides and RBCs are by far more abundant than the white

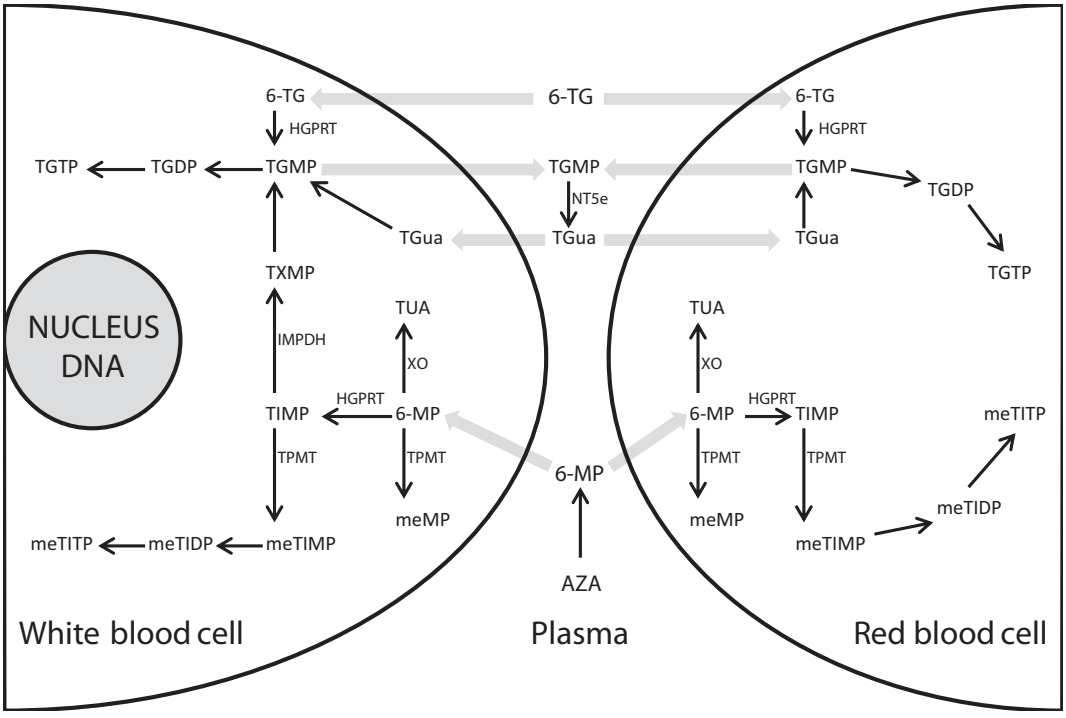

Figure 1 Simplified scheme of thiopurine metabolism. 6-MP, 6-mercaptopurine; 6-TG, thioguanine; ABCC4, multidrug resistance-associated protein 4, HGPRT, hypoxanthine-guanine phosphoribosyltransferase; IMPDH, inosine monophosphate dehydrogenase; meMP, methylmercaptopurine; meTIDP, methylthioinosine diphosphate; meTIMP, methylthioinosine monophosphate; meTITP, methylthioinosine triphosphate; NT5e, ecto-5-nucleotidase; TGDP, thioguanosine diphosphate; TGMP, thioguanosine monophosphate; TGTP, thioguanosine triphosphate; TIMP, thioinosine monophosphate; TGua, thioguanosine; TPMT, thiopurine methyltransferase, TUA, thiouric acid; TXMP, thioxanthine monophosphate; XO, xanthine oxidase.