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Alimentary Tract

Thiopurine methyltransferase activity combined with 6-thioguanine metabolite levels predicts clinical response to thiopurines in patients with inflammatory bowel disease☆

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Abstract

Background/aims. 6-Mercaptopurine and its prodrug azathioprine are effective for the treatment of inflammatory bowel disease. Thiopurine methyltransferase is important for the metabolism of thiopurines. However, there is controversy as to the clinical utility of measuring thiopurine methyltransferase enzyme activity and 6-thioguanine nucleotide levels. Our aim was to determine if thiopurine methyltransferase enzyme activity and 6-thioguanine nucleotide level monitoring would predict response to therapy with thiopurines in patients with inflammatory bowel disease.

Methods. Baseline thiopurine methyltransferase enzyme activity prior to initiation of therapy with either 6-mercaptopurine or azathioprine was determined in 39 patients with inflammatory bowel disease. The association between clinical response and thiopurine methyltransferase activity and 6-thioguanine nucleotide levels singly or in combination were analysed.

Results. Seventeen of 39 patients (44%) responded to 6-mercaptopurine or azathioprine therapy. Thiopurine methyltransferase enzyme activity below the mean of 30.5 U was significantly associated with clinical response. The thiopurine methyltransferase low phenotype was associated with response in 65% vs. 29% in individuals with thiopurine methyltransferase enzyme activity above 30.5 U ($p = 0.05$). There was no correlation between thiopurine methyltransferase activity and 6-thioguanine nucleotide levels. The maximal 6-thioguanine nucleotide levels did not predict clinical response. When combining thiopurine methyltransferase enzyme activity and 6-thioguanine nucleotide levels, the combination of thiopurine methyltransferase low/6-thioguanine nucleotide high was associated with response in 7/7 (100%) vs. only 2/8 (25%) with the combination of thiopurine methyltransferase high/6-thioguanine nucleotide low ($p = 0.01$).

Conclusions. Thiopurine methyltransferase activity inversely correlated with clinical response to thiopurine treatment in inflammatory bowel disease. Thiopurine methyltransferase enzyme activity below 30.5 U combined with a post-treatment 6-thioguanine nucleotide level >230 pmol/8 $\times 10^8$ erythrocytes was the best predictor of response.

Keywords: Crohn's disease; Inflammatory bowel diseases; Metabolite monitoring; Thiopurines; Ulcerative colitis

1. Introduction

6-Mercaptopurine (6-MP) and its pro-drug azathioprine (AZA) are often used for the treatment of moderate to severe inflammatory and fistulizing Crohn's disease, the prevention of postoperative CD recurrence and in steroid-dependent CD or ulcerative colitis (UC) [1–4]. Treatment with 6-MP and AZA on a standard weight based dosing regimen, however, results in variable efficacy limited by inter-individual vari-

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ability in drug tolerance and thiopurine methyltransferase (TPMT) catalysed thiopurine metabolism.

AZA is nonenzymatically converted to 6-MP after oral administration, which then undergoes metabolism by three main competing enzymatic pathways catalysed by xanthine oxidase, hypoxanthine guanine phosphoribosyl transferase (HPRT), and TPMT to produce active 6-thioguanine nucleotides (6-TGNs), inactive thiouric acid, methylated thioinosine monophosphate (me-TIMP), and methylated mercaptopurine metabolites (6MMP and 6MMPR) [2,5]. 6-TGNs (6-TGMP, 6-TGDP, and 6-TGTP) exert their cytotoxic and therapeutic immunomodulating effects by interfering with nucleic acid synthesis as they accumulate intracellularly [6]. It is proposed that 6-TGTP nucleotides signal lymphocyte apoptosis [7] and the accumulation of 6-TGNs may inhibit proinflammatory mediated effects [8]. Variable TPMT activity results in differential production of 6-TGN and 6MMPR metabolites with lower TPMT activity shunting the metabolic pathway towards production of 6-TGNs [9].

Genetic polymorphisms of TPMT are responsible for the variable expression of enzyme activity translating into variable efficacy and toxicity. There is a trimodal distribution of TPMT genotype [10] and more than 10 different mutant variant alleles that are associated with low TPMT enzyme activity levels (TPMT *2 to *18) have been described [11,12]. Those with heterozygous and homozygous variant allele genotypes express lower or null TPMT activity, respectively, with significant risk for toxicity on a standard weight based dosing regimen and may need therapeutic dose adjustments [13]. Interestingly, most patients who acquire leucopenia after initiation of thiopurine treatment have a normal TPMT genotype [1,9,14–16].

Measurement of 6-TGN and 6MMPR metabolites is often used to predict clinical response and toxicity to thiopurines. A higher level of 6-TGN alone (>235 pmol/8 $\times$ 10^8 RBC) has been associated with clinical response, but higher risk of leucopenia and infection [1,9]. 6MMP levels >5700 pmol/8 $\times$ 10^8 RBC have been associated with risk for hepatotoxicity [9]. There is, however, no consensus regarding the clinical utility of therapeutic monitoring as predictors of response in inflammatory bowel disease (IBD) patients treated with thiopurines [17–19].

It is also unclear if TPMT enzyme activity levels are predictive of clinical response or toxicity in patients with IBD. Several studies have previously investigated TPMT enzyme activity as a predictor of response to thiopurine therapy, toxicity, and the need for thiopurine dose adjustments [9,15,17,19–23]. The most recent study by Cuffari et al. [21], demonstrated that TPMT activity below the mean for their population had higher levels of 6-TGN metabolite levels and lower TPMT activity was predictive of response to thiopurine therapy for adult IBD patients. This is consistent with the findings of Ansari et al. [17] and Campbell et al. [20] who showed that higher TPMT activity diminished the likelihood of clinical response and low or intermediate TPMT activity was associated with adverse effects. Dubinsky et al. [9], has previously shown that an intermediate TPMT enzyme activity was predictive of higher 6-TGN metabolites, which in turn was significantly associated with clinical response in paediatric and adult IBD patients. However, there has been contradictory results obtained by subsequent studies trying to correlate TPMT enzyme activity with 6-TGN levels or clinical response [15,19,23].

In the current study we revisited the association of TPMT activity level before initiation of thiopurine treatment combined with 6-TGN metabolite level monitoring during treatment to predict clinical response or toxicity to thiopurines in patients with IBD.

2. Materials and methods

2.1. Patients

A database of 71 patients with established clinical diagnosis of CD or UC with known baseline TPMT enzyme activities determined prior to initiation of standard weight based dosing of 6-MP or AZA was reviewed. Thirty nine patients were included in this study who met eligibility criteria of treatment duration of at least 6 months on 6-MP or AZA and the availability of the treating physician's clinical assessment of disease activity both at the beginning and endpoint of treatment evaluation at our IBD centre. Six individuals were excluded due to early withdrawal of treatment for drug intolerance, leucopenia, or hepatotoxicity prior to six months clinical observation on 6-MP or AZA. One additional patient was excluded due to initiation of concomitant infliximab therapy within 6 months of AZA treatment initiation. Sufficient clinical follow up data were unavailable in 25 patients. These patients, after initial consultation at our IBD Center, were referred back to their treating gastroenterologists for initiation and monitoring of thiopurine treatment. The study received approval from the Cedars-Sinai IRB (protocol # 8589).

This study was a retrospective review of data collected by a single reviewer (L.Y.K.) blinded to the predetermined baseline TPMT activity. Routine patient demographics were recorded, including age, gender, and weight. Initial and maximum treatment doses were recorded. AZA doses were converted by a factor of 2.08 for equivalent comparison of drug bioavailability. The use of corticosteroids with dosage, and the use of aminosalicylates were recorded. Disease duration, location, and characteristics including extraintestinal manifestations, stenosis, fistulas, and the need for surgical interventions were recorded. Disease activity was determined by Harvey–Bradshaw index (HBI) and simple clinical colitis activity index (SCCAI) scores for Crohn's disease and ulcerative colitis, respectively [24,25].

Standard weight based dosing of immunomodulators was followed and adjusted by the treating physician as tolerated by the patient to achieve a 6-MP dose concentration of 1.0–1.5 mg/kg/day and AZA dose concentration of