Research Article

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Characterisation and utility of thiopurine methyltransferase and thiopurine metabolite measurements in autoimmune hepatitis

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Background & Aims: Corticosteroids alone or in conjunction with azathioprine (AZA) is the standard treatment in autoimmune hepatitis (AiH). Individual variations in thiopurine (TP) metabolism may affect both drug efficacy and toxicity. Our aim was to investigate the utility of thiopurine methyltransferase (TPMT) as well as thioguanine nucleotide (TGN) and methylthioinosine monophosphate (meTIMP) metabolite measurements with regard to clinical outcome.

Methods: Two hundred thirty-eight patients with AiH were included in this cross-sectional study. TPMT status was assessed in all patients, while TGN and meTIMP were measured in patients with ongoing TP medication. Clinical outcome was evaluated by liver tests and the ability to withdraw steroids.

Results: TPMT genotyping ($n = 229$) revealed 207 (90.4%) wild-type and 22 heterozygous patients. One hundred forty-three patients had ongoing TP therapy with AZA ($n = 134$) or mercaptopurine (MP; $n = 9$); response was judged as complete response (CR) in 113 patients and partial response (PR) in 30 patients. Both TP dose (1.64 vs 1.19 mg/kg; $p = 0.012$) and TPMT activity (14.3 vs 13.5; $p = 0.05$) were higher in PR, resulting in similar TGN levels (PR: 121 pmol/8×10^8 red blood cells [RBC]; CR: 113 pmol/8×10^8 RBC; $p = 0.33$) but higher meTIMP levels in PR (1350 vs 400 pmol/8×10^8 RBC; $p = 0.004$). Patients able to withdraw steroids or who were using $\leqslant 5$ mg prednisolone daily were treated with lower TP doses than patients on higher steroid doses (1.15 vs 1.18 vs 1.82 mg/kg; $p < 0.001$).

Conclusions: TP metabolite measurements are of clinical value in AiH patients who do not respond to standard TP treatment and for the identification of a shifted metabolism, which may demand an alternative treatment strategy.

Keywords: Autoimmune hepatitis; Thiopurines; Thiopurine methyltransferase; Thiopurine metabolites; Drug monitoring.

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Abbreviations: AZA, azathioprine; AiH, autoimmune hepatitis; TP, thiopurine; TPMT, thiopurine methyltransferase; TGN, thioguanine nucleotide; meTIMP, methylthioinosine monophosphate; CR, complete response; PR, partial response; RBC, red blood cells; MP, mercaptopurine; IBD, inflammatory bowel disease; BW, body weight; NS, no steroids; LDS, low-dose steroids; MDS, medium-dose steroids; AE, adverse event; GI, gastrointestinal; ANA, anti-nuclear antibodies; SMA, smooth muscle antibodies; AMA, antimitochondrial antibodies; LKMA, liver–kidney microsome antibodies; PSC, primary sclerosing cholangitis; PBC, primary biliary cirrhosis; ALT, alanine aminotransferase; MMF, mycophenolate mofetil; Ltx, liver transplantation.

Introduction

Autoimmune hepatitis (AiH) is an unresolving inflammation of the liver that, if not treated, has a high mortality rate. Studies in the 1970s established that prednisolone—alone or in combination with azathioprine (AZA)—was an effective treatment for AiH, with clinical remission rates of more than 80% [1–3]. Subsequently, it was shown that AZA alone was an effective treatment option for maintaining clinical remission in AiH [4,5]. Nevertheless, a significant proportion of patients with AiH remain on corticosteroids because of AZA toxicity, insufficient treatment response to AZA monotherapy, or perhaps subtherapeutic thiopurine (TP) doses [5,6].

The TP drugs AZA and mercaptopurine (MP) are metabolised by enzyme systems that exhibit interindividual genetic variations, affecting both tolerability and efficacy during treatment. AZA is converted to MP, which after further metabolism results in the formation of active thioguanine nucleotides (TGN) [7]. The TGN metabolites act as purine antagonists and induce cytotoxicity and immunosuppression by inhibition of RNA, DNA, and protein synthesis [8]. They also induce apoptosis in activated T cells via *Rac-2* inhibition [9]. MP is methylated by the enzyme thiopurine methyltransferase (TPMT), which leads to the production of methylthioinosine monophosphate (meTIMP). This metabolite is found in concentrations that far exceed TGN concentrations [10] and is a potent inhibitor of purine de novo synthesis in vitro [11]. Genetic polymorphisms in the *TPMT* gene

(*TPMT*2* to *25*) are associated with decreased TPMT activity [12–16] and the development of myelotoxicity resulting from high TGN metabolite concentrations [17].

Measurement of TGN levels has been suggested to be helpful in determining treatment intensity and to optimize TP treatment in inflammatory bowel disease (IBD) [18–20]. A number of patients with unresponsive disease who had their AZA dosage increased went into remission while their median TGN levels increased [17]. Other patients failed therapy and were characterised by suboptimal TGN levels and preferential production of methylated metabolites (i.e., meTIMP) upon dose escalation [21]. TPMT testing has not proven predictive for the emergence of TP-related toxicity in AiH [22–24], and whether the AZA dosage and determination of TP metabolites is of importance in AiH is unclear. Although two small studies in children with AiH concluded that TP metabolite measurements were helpful in optimizing TP dosing and identifying noncompliance as well as medication toxicity [6,25], one study in adults with AiH reported similar TP metabolite levels regardless of whether steroids were needed to maintain remission [23].

The primary aim of this study was to investigate the utility of TPMT and TP metabolite measurements in a large population of patients with AiH with regard to clinical outcome.

Patients and methods

The Swedish Internal Medicine Liver Club is a collaboration of colleagues from all university hospitals in Sweden aiming to perform studies of various liver diseases.

Patients

This cross-sectional study was performed in unselected patients with AiH seen at seven university hospitals (Karolinska/Huddinge, Linköping, Malmö, Lund, Sahlgrenska/Göteborg, Östra/Göteborg, and Umeå) between September 2005 and October 2006. AiH score calculations were performed according to the International Autoimmune Hepatitis Group criteria with minor modifications (absence of histocompatibility leukocyte antigen status; varying histology description) [26]. Only patients with a probable or definitive diagnosis of AiH (i.e., post-treatment AiH scores >11) were included. An overlap syndrome was suspected in 31 (13%) cases at some time during follow-up. The clinical diagnosis was that 21 AiH patients had an overlap with primary sclerosing cholangitis (PSC), and 10 AiH patients had an overlap with primary biliary cirrhosis (PBC). In half of these patients (15 of 31, 48%) the diagnosis was based on strict histologic criteria, and these patients have been included in two recent publications from our group [27,28]. For the remaining 16 patients, an overlap syndrome was suspected on a combined evaluation of laboratory parameters, presence of autoantibodies, and liver biopsy findings.

Outcome was characterised in three categories: (1) complete response (CR) as a normalisation of transaminase levels; (2) partial response (PR) at transaminase levels below five times the upper limit of the normal range but without normalisation; and, (3) no response at transaminase levels above five times the upper limit of normal. Informed consent was obtained from all patients. The study was approved by the ethics committees at the respective hospitals.

Methods

TPMT activity and genotype were measured in all patients, and the TP metabolites TGN and meTIMP were measured in patients with ongoing TP therapy. TPMT activity was determined with a well-established high-performance liquid chromatography (HPLC) method [29]. Briefly, we measured the formation of 6-methylmercaptopurine from MP with radiolabelled *S*-adenosyl-L-methionine used as the methyl donor. Product formation was measured by a liquid scintillation counter. One unit of enzyme activity represents the formation of 11 nmol of 6-methylmercaptopurine per millilitre of red blood cells (RBC) per hour of incubation. The interassay and intra-assay coefficients of variation are 4.7% and 3.3%, respectively.

TGN and meTIMP were determined as previously described [29]. Blood collected in ethylenediaminetetraacetic acid tubes were centrifuged, and RBC were washed and diluted in saline to a final concentration of 8×10^8 cells per 200 μL prior to storage at −70 °C. TGN and meTIMP were then determined by reverse-phase HPLC at 330 nm as purine bases after acid hydrolysis and an extraction procedure. The limit of quantification for TGN was 20 pmol/8×10^8 RBC and for meTIMP 300 pmol/8×10^8 RBC. At these levels, the interassay coefficients of variation are 12.2% and 17.4%, respectively.

Normal TPMT activity was defined as ⩾9.0 U/mL of RBC, intermediate TPMT activity as 2.5–8.9 U/mL of RBC, and low TPMT activity as <2.5 U/mL of RBC. Using these cut-off levels for TPMT activity, we have previously found a close relationship between TPMT phenotype and genotype [30]. TPMT genotype was determined by a pyrosequencing method previously described [12]. The patients were genotyped for the following nucleotide substitutions: 238G > C, 460G > A, 719A > G, 292G > T, intron IX/exon X splice site (G > A), 146T > C, 539A > T, 681T > G, 644G > A, and 430G > C (*TPMT*2, *3A, *3B, *3C, *3D, *4, *5, *6, *7, *8,* and *10*). Genotyping for +1A > G (*TPMT*14*) and for IVS7-1G > A (*TPMT*15*) was performed as previously described [12]. However, for *TPMT*15*, polymerase chain reaction primers for exon VIII were used [30].

Statistics

Results are presented as medians with interquartile ranges. Differences between groups were analysed by using the Mann–Whitney test or the Kruskal–Wallis test. The Spearman rank order correlation coefficient, ρ_s, was applied to test correlations between parameters. MP doses were recalculated into AZA equivalent doses by a conversion factor of 2.08 [31]. Statistical analysis was performed using the Statistical Package for the Social Sciences v.14.0 for Windows (SPSS, Chicago, IL, USA).

Results

Of the 238 patients, 143 had ongoing TP therapy, 32 had discontinued TP therapy, and the remaining 63 patients had never been treated with TP. Details regarding medication can be seen in Fig. 1, and patient characteristics are detailed in Table 1.

Thiopurine methyltransferase activity

TPMT activity displayed a bimodal distribution, with 20 patients (10.5%) having activity <9.0 U/mL of RBC and the remaining activity in the normal range. TPMT genotype was assessed in 229 patients (96%), of which 207 (90.4%) had a *1/*1 (wild-type) genotype, 20 patients had a *1/*3A genotype, and 2 had a *1/*3C genotype. No patient displayed homozygosity for TPMT deficiency. In nine patients without TPMT genotyping, two had TPMT activity in the intermediate range (6.8 and 7.4 U/mL of RBC, respectively), and seven patients had normal activity between 11.9 and 17.2 U/mL of RBC.

There was an incomplete concordance between TPMT genotype and phenotype in five patients. Two heterozygote patients, both on therapy, had TPMT activity levels ⩾9.0 U/mL of RBC (9.0 and 9.9, respectively); three wild-type patients, two of them without therapy, had TPMT activity levels in the range of 7.8 to 8.0 U/mL of RBC.

Thiopurine dose and metabolites

There was no difference in the TP dose between patients with intermediate and normal TPMT activity (1.1 [range: 0.6–1.5] vs 1.3 [range: 0.9–1.7] mg/kg of body weight [BW]; $p = 0.27$). Patients with normal TPMT activity had lower TGN (112 [range: 70–161] vs 206 [range: 130–353] pmol/8×10^8 of RBC; $p = 0.01$) and higher meTIMP (600 [range: 299–1500] vs 0 [range: