Journal of Crohn's and Colitis, 2015, 676–679
doi:10.1093/ecco-jcc/jjv086
Advanced Access publication May 12, 2015
Short Report

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Successful Mercaptopurine Usage despite Azathioprine-Induced Pancreatitis in Paediatric Crohn's Disease

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Abstract

Background: Azathioprine [AZA] and mercaptopurine [MP] are recommended for maintenance of steroid-free remission in children with Crohn`s disease [CD]. Azathioprine-induced pancreatitis, an idiosyncratic and major side effect, has been considered as an absolute contraindication for the use of a second thiopurine in IBD patients.

Materials and Methods: We describe two children with CD in whom MP were successfully trialled after a confirmed azathioprine-induced pancreatitis, being well tolerated in both cases.

Results: Two boys [13 and 10 years old] started exclusive enteral nutrition after diagnosis of moderate (Pediatric Crohn´s Disease Activity Index [wPCDAI] = 45) and mild [wPCDAI = 35] CD. Both developed an acute mild to moderate pancreatitis after 2 and 3 weeks, respectively, of AZA treatment but recovered fully in hospital after AZA withdrawal. They started on MP treatment without any adverse effect. They were tested for the presence of polymorphisms 238G>C, 460G>A, and 719A>G in the TPMT gene and 94C>A and 21>C in the ITPase. Both patients were wild-type for all tested polymorphisms.

Conclusions: Azathioprine-induced acute pancreatitis should not be considered as an absolute contraindication for the use of MP. Further investigation is required to create a better understanding of the mechanism underlying the adverse events and to allow more possibilities for personalised therapy.

Keywords: Thiopurines; azathioprine; mercaptopurine; pancreatitis; Crohn´s disease; children

1. Introduction

Thiopurines, azathioprine [AZA], or mercaptopurine [MP] are recommended as one option for maintenance of steroid-free remission in children with Crohn's disease at risk for poor disease outcome.[1] The most effective daily dose appears to be 2.0–2.5 mg/kg for AZA and 1.0–1.5 mg/kg for MP.[2]

The use of thiopurines has been associated with two types of side effects: dose-independent reactions, which occur within weeks

following administration of the drug, and dose-related side effects, which are thought to be related to the intracellular concentration of thiopurine metabolites.[3] The different thiopurines' adverse effects can be classified as major [pancreatitis, neutropenia, hepatotoxicity, and malignancy] and minor (gastrointestinal [GI] disturbance, flu-like illness, rash, etc.)[4,5,6] Thiopurine-induced pancreatitis occurs in up to 5% of patients and is considered an idiosyncratic, dose-independent drug reaction.[7,8]

AZA is a pro-drug and requires conversion to active metabolites. When absorbed, AZA reacts with reduced glutathione, leading to the formation of MP and a nitroimidazole conjugate of reduced glutathione. Although this reaction can occur spontaneously, the presence of the enzyme glutathione-S-transferase [GST] can accelerate this reaction.[9] One of the mechanisms that could explain the observed higher incidence of azathioprine-induced adverse effects in patients with the GST-M1 normal genotype is that a high GST-M1 activity could result in higher consumption of glutathione, leading to higher cellular and tissue damage.[3] After MP formation, there are three possible enzymatic pathways: thiopurine methyltransferase [TPMT], xanthine oxidase [XO], and hypoxanthine phosphoribosyltransferase [HPRT]. TPMT metabolises MP into an inactive metabolite 6-methylmercaptopurine [6MMP], whose levels are associated with hepatotoxicity. The XO pathway leads to the production of 6-thiouric acid, an inactive metabolite excreted in urine. HPRT followed by inosine monophosphate dehydrogenase [IMPDH] and guanosine monophosphate synthase [GMPS] leads to production of the 6-thioguanine nucleotides [6-TGN], the active metabolite. High erythrocyte 6-TGN concentrations have been associated with an increased risk of dose-dependent adverse events, like myelotoxicity.[10,11] Quantification of TPMT activity has been used to identify patients at risk of myelotoxicity.[12]

Other authors have studied the relationship between low activity of inosine triphosphate pyrophosphatase [ITPase], which catalyses the pyrophosphohydrolysis of inosine triphosphate to inosine monophosphate, and occurrence of adverse events.[13]

Azathioprine-induced pancreatitis, an idiosyncratic and major side effect, has often been considered as an absolute contraindication for the use of a second thiopurine in IBD patients, whereas other studies have suggested trying MP in these patients under close surveillance.[4]

2. Materials and Methods

This report presents 2 cases in which MP was successfully used in children with CD, following a previous episode of confirmed azathioprine-induced pancreatitis. TPMT levels [high-performance liquid chromatography, Cerbá Laboratory, Barcelona] were measured before starting AZA. Total genomic DNA was extracted from buccal swabs using a commercial kit [QIAamp® DNA Investigator Kit, QIAGEN] according to the manufacturer's recommendations. Individuals were genotyped at the *TPMT*3A* [G460A and A719G], *TPMT*3B* [G460A; Ala154→Thr, rs1800460], and *TPMT*3C* [A719G; Tyr240→Cys, rs1142345] alleles by polymerase chain reaction–restriction fragment length polymorphism [PCR-RFLP]-based protocol, using previously described primers.[14] *TPMT*2* allele [G238C; Ala80→Pro, rs1800462] was genotyped by real-time PCR, using commercial kit [TaqMan® SNP Genotyping Assay, Life Technologies] according to the manufacturer's recommendations. Two polymorphisms on *ITPA* gene [94C>A; Pro32→Thr, rs1127354] and [IVS2+21A>C; rs7270101] were genotyped by PCR-RFLP based protocol, using previously described primers.[15] The PCR-RFLP products were separated by 3% agarose electrophoresis. Positive and negative controls were available for each genotype.

3. Results

3.1. Case 1

This boy was diagnosed with CD at 13 years of age using standard investigations.[16] His phenotype was A1bL3L4aB1G1p according to the Paris classification.[17] He had a 4-month history of fatigue and weight loss and 1 week of diarrhoea. The wPCDAI[18] at diagnosis was 45 and TPMT activity 19U/l [normal range 5.0–40.0 U/ml]. He started treatment to induce remission with exclusive enteral nutrition [EEN], metronidazole and azithromycin and, after 2 weeks of EEN maintenance, treatment with AZA was started [2.5 mg/kg/day]. Amylase and lipase were both in the normal range before AZA treatment. After 3 weeks on AZA therapy, when in clinical remission, he developed abdominal pain and vomiting and was hospitalised. Analyses showed increased levels of amylase [934U/l, normal range 25–115 U/l] and lipase [6549U/, normal range 65–230 U/l]. An abdominal ultrasound showed widespread pancreatic enlargement, most prominent in the head, with homogeneous echostructure. AZA was stopped and the patient fully recovered over the following 5 days. After 2 weeks, the patient was started on MP [0.1 mg/kg/day with a subsequent increase to 1.5 mg/kg/day] and corticosteroids. Lipase and amylase levels were normal during 15 months of follow-up. The patient was wild-type for all tested polymorphisms.

3.2. Case 2

This 10 year-old boy was diagnosed with CD A1bL3L4bB1G1 according to the Paris classification. He reported a history of 10 months of bloody diarrhoea, fatigue, and abdominal pain. The wPCDAI at diagnosis was 35 and TPMT activity 18.6 U/l [normal range 5.0–40.0 U/ml]. After 2 weeks of EEN he started treatment with AZA at a dose of 2.5 mg/kg/day. Two weeks later he developed epigastric pain, fever, and increase of lipase levels to 536 U/l [normal range 65–230 U/l] and was hospitalised. An abdominal ultrasound showed diffuse pancreatic enlargement with more marked swelling of the pancreatic head, alteration of the peripancreatic fat, and a moderate amount of free fluid in the pelvis. AZA was ceased immediately. He required parenteral nutrition for 48 h, with good outcome. After 2 weeks he began treatment with MP at 0.5 mg/kg/day which was increased to 1.4 mg/kg/day, with no adverse effects during 14 months of follow up. The patient was also wild-type for all tested polymorphisms.

4. Discussion

Azathioprine-induced acute pancreatitis should not be an absolute contraindication for the use of thiopurines. In these cases of azathioprine-induced pancreatitis, administration of MP can be a good alternative rather than immediately withdrawing all thiopurines.[7]

Beswick *et al*. described[19] 64 patients with IBD over 10 years, of whom 23 were intolerant to AZA, seven of whom developed acute pancreatitis. Three of these seven patients went on to tolerate MP. Ledder *et al.* described four paediatric patients with CD who developed AZA-induced pancreatitis, and a subsequent trial of MP was tolerated in all four patients.[20] Azathioprine-induced pancreatitis is more prevalent in children with CD [4,9%] than in patients with ulcerative colitis [1.1%] or autoimmune hepatitis [1.5%].[21] There are no consistent clinical data to predict which patients on AZA will develop pancreatitis and tolerate MP. Beswick *et al*.[19] did not find

differences between those patients who did and did not tolerate MP with respect to age, gender, disease distribution, or initiation timing. Switching to MP is reported to be less successful if the reason for AZA intolerance is flu-like illness or pancreatitis.[4]

As previously described in the literature, in our patients pancreatitis occurred within the first 4 weeks after starting AZA and resolved after AZA was withdrawn.

It is unclear whether the incidence of AZA-induced pancreatitis is greater in patients with CD than in those treated with AZA for other diseases. Some studies found a higher risk in CD than in patients with ulcerative colitis.[8,22] This difference could be explained by the occurrence of pancreatitis as an extraintestinal manifestation of CD. Van Geenen *et al.* found no differences between the cumulative incidence of thiopurine-induced pancreatitis in CD compared with patients with vasculitis or ulcerative colitis.[23]

Some authors consider TPMT activity as a good predictor of which patients have a higher risk of developing serious adverse events, especially dose-dependent, as it is a major determinant factor in the inactivation of AZA metabolites. However, other authors have reported that TPMT genotype/phenotype does not predict myelotoxicity in IBD patients treated with thiopurines.[24,25] Due to the many factors that influence the metabolism of thiopurines, frequent blood testing should still be performed in IBD patients on treatment with thiopurines irrespective of the activity of TPMT.

Although the enzyme TPMT has been the most extensively studied, several factors not related to TPMT activity may be responsible for thiopurine-induced pancreatitis. Deficient ITPase activity may increase the risk of pancreatitis[26] as well as the presence of 837C>T polymorphism in XO.[27] The role of GST in azathioprine-induced pancreatitis is under investigation and published data showed that GST-M1 null genotype is less frequent in patients that developed an adverse event and are less sensitive to AZA therapy.[3,28] However, more data are needed to confirm an association of presence of this genotype with pancreatic toxicity.[3]

Heap *et al.* found an association between HLA-DQA1*02:01–HLA-DRB1*07:01 haplotype [rs2647087] and increased risk of developing pancreatitis: 9% risk in patients who were heterozygotes and 17% in homozygotes.[29] Despite an increased understanding of thiopurine metabolism, the role of metabolite testing in clinical practice continues to be debated. Further investigations are required to improve understanding of the mechanism underlying the adverse events and to gain efficacy and decrease toxicity by optimised drug monitoring and individualisation of therapy.

In conclusion, azathioprine-induced pancreatitis should not be considered as an absolute contraindication for future use of MP in CD children. Genotyping of polymorphisms in enzymes involved in the metabolic pathway of thiopurines as well as recently identified variants in the HLA-region [rs2647087] can further help to determine the optimal dosing of these drugs and the possibility of adverse events.

Acknowledgments

Thank you to David Wilson and Johan Van Limbergen for their contributions to this manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Author contributions

All authors have made substantial contributions to all of the following: [1] the concept and design of the study and analysis and interpretation of data, [2] drafting the article and revising it critically for important intellectual content, [3] final approval of the version to be submitted. MK, LB, and KL performed genetic analysis and drafted that specific part of the article. This manuscript, including related data, figures and tables has not been previously published and is not under consideration elsewhere.

Funding

No specific funding had been received for this work. The data have been generated as part of the routine work of both implicated organizations.

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