CASE REPORT

Safe treatment of thiopurine S-methyltransferase deficient Crohn's disease patients with azathioprine

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Thiopurine S-methyltransferase (TPMT) deficient patients develop life threatening haematotoxicity (for example, pancytopenia) when treated with a standard dose of azathioprine (AZA) and 6-mercaptopurine (6-MP) due to excessive accumulation of cytotoxic metabolites. At present, it is generally recommended that these patients should not receive AZA or 6-MP treatment for inflammatory bowel disease. We report for the first time that Crohn's disease patients with TPMT deficiency can be successfully treated with AZA. We illustrate this with three cases where treatment has been successful and toxicity has been avoided by carefully titrating the drug dose. Thus very low TPMT activity demands pharmacogenetically guided *dosing*.

It is well recognised that immunomodulatory treatment with azathioprine (AZA) or 6-mercaptopurine (6-MP) even at standard doses can cause serious and fatal adverse effects in patients with thiopurine S-methyltransferase (TPMT) deficiency and inflammatory bowel disease (IBD).[1 2] In various populations studied, approximately 0.3% of subjects have a much reduced TPMT activity. In these patients, as no methylated metabolites are formed, an increased proportion of thiopurine prodrug substrate is metabolised to pharmacologically active 6-thioguanine nucleotides (6-TGN), leading to toxic accumulation after standard dosages. Severe myelotoxicity, which may be fatal, ensues. Therefore, IBD patients with TPMT deficiency are usually denied the benefit of thioguanine based medication.[3]

However, as there are no therapeutic alternatives, TPMT deficient children with acute lymphoblastic leukaemia (ALL)[4–6] have been treated successfully with these drugs for maintenance of remission. Immediate drug related toxicity was avoided by greatly reducing the dose.

We have identified two TPMT deficient patients who were restarted on very low dose AZA after severe leukopenia or pancytopenia. A third TPMT deficient patient on AZA was found to be in remission without ever having experienced adverse effects. To our knowledge, these are the first reports of successful treatment of TPMT deficient IBD patients with thiopurines.

CASE REPORTS

Case No 1

A 32 year old man diagnosed with Crohn's disease aged 26 (Vienna classification proposal[7] A1) was shown to have terminal ileitis and colitis (L3) as well as enteric fistulae (B3) in May 1992. He was started on 1.3 mg/kg body weight AZA daily. Eight weeks later, he was admitted for severe myelosuppression (haemoglobin 3.8 g/dl; leucocytes 3400 /mm^3 (nadir 1270/mm^3); thrombocytes 17 000/mm^3) and AZA therapy was stopped. Full blood count normalised after three weeks and the patient was discharged. Subsequently, his disease was poorly controlled with various doses of oral steroids. In January 1996, he had a descending colostomy and resection of enteric fistulae. Three months later he was restarted on 0.29 mg/kg body weight AZA daily when his abdominal symptoms recurred and a new arthropathy developed despite steroids. Two months later, on endoscopy, he was free of inflammation and a re-anastomosis of the colostomy was successfully performed. He remained on 0.29 mg/kg body weight AZA for a further five months. Repeat full blood counts were normal. TPMT activity in red blood cells (RBC) was measured, as described previously,[8] and was found to be very low (2 nmol 6-methylthioguanine [6-MTG]/g Hb/h) which reflects the patient's homozygous mutant TPMT genotype (**3A/*3A*).[9]

Case No 2

A 21 year old woman with a six year (A1) history of histologically proven Crohn's disease (inflammation (B1) in the terminal ileum and caecum (L3)) was started on 1 mg/kg body weight AZA in September 1997 after two flare ups while tapering methylpredisolone. After four weeks, when the AZA dose was increased to 1.5 mg/kg body weight, mild leukopenia (2510/mm^3) was noticed and the dose was reduced to 1 mg/kg body weight. During the following year the patient remained in remission. In March 1999, when she developed tonsillitis with moderate leukopenia (3130/mm^3), AZA was stopped and the patient recovered. In August 1999, methylprednisolone was restarted together with AZA. In view of the repeated episodes of haematological side effects while on standard dosages, a much lower dose of 12.5 mg (0.25 mg/kg body weight) AZA was given daily. When measured three weeks later, the concentration of 6-TGN in RBC was unexpectedly high (1014 pmol/8×10^8 RBC). AZA was reduced to 10 mg/day and repeat measurements demonstrated 6-TGN had decreased (679 pmol/8×10^8 RBC). Methylprednisolone was subsequently replaced by 9 mg budesonide daily. A further four months later, in May 2000, the patient received a single infusion of infliximab (5 mg/kg body weight) because of a flare up. Since then she has been in continuous remission for more than 12 months on just 8 mg (0.16 mg/kg body weight) AZA and 9 mg budesonide daily without adverse effects. TPMT activity was found to be very low (< 2 nmol 6-MTG/g Hb/h) which is in keeping with her homozygous mutant TPMT genotype (**3A/*3A*).

Abbreviations: 6-MP, 6-mercaptopurine; 6-TGN, 6-thioguanine nucleotides; ALL, acute lymphoblastic leukaemia; AZA, azathioprine; IBD, inflammatory bowel diseases; RBC, red blood cells; TPMT, thiopurine S-methyltransferase; TIMP, 6-thioinosine-5′-monophosphate; 6-MTG, 6-methylthioguanine.

Case No 3

In October 1993, a 22 year old man (B2, L3), who had been diagnosed with Crohn's disease 10 years previously, was started on 0.71 mg/kg body weight AZA. Subsequently, steroids were gradually tapered off and, in September 1994, completely withdrawn. The patient remained asymptomatic on 0.71 mg/kg body weight AZA daily. Leucocyte count was in the range 3600 to 5400/mm^3 except for two short episodes (2800 and 2900/mm^3). Phenotyping revealed TPMT deficiency in April 2000. Thereafter, 6-TGN levels were measured repeatedly (748–1144 pmol/8×10^8 RBC) with methylated metabolites being undetectable. After AZA dose adjustment to just 0.26 mg/kg body weight daily, 6-TGN levels were somewhat lower (797 and 884 pmol/8×10^8 RBC). The patient remained well. TPMT activity was confirmed to be very low (<2 nmol 6-MTG/g Hb/h). Genotyping revealed compound heterozygous TPMT alleles (**3A/*3C*).

DISCUSSION

TPMT is a pharmacogenetic polymorphism which should be routinely considered to safely and effectively administer thiopurines in whatever medical specialty they are employed. Prospective pheno-/genotyping of TPMT has become standard practice in major cancer treatment centres such as the Mayo Clinic (Rochester, Minnesota, USA) and St Jude's Children's Research Hospital (Memphis, Tennessee, USA).[11] The concept of pharmacogenetic based medicine has been proposed recently.[10 12]

The cases presented demonstrate that TPMT deficiency in patients with IBD per se does not preclude thiopurine therapy and hence offers a further therapeutic option for this group of patients. It illustrates what could be gained from prospective screening for *TPMT* polymorphism. Furthermore, the cases presented prove that methylated metabolites are not essential for the therapeutic action of thiopurines in Crohn's disease.

In patients who finally respond to thioguanines, it has been shown that an increase in 6-TGN levels correlates with clinical response and consequently a target concentration of 6-TGN level could be used as a therapeutic guide for patients on AZA or 6-MP.[13 14] As a large number of patients already respond to 2 mg/kg AZA (or 1 mg/kg 6-MP) despite 6-TGN levels lower than the proposed threshold, this may be mainly applicable to patients not responding to standard doses.[15]

On the assumption that 6-TGNs are the main active compounds in IBD, TPMT deficient patients should, if anything, be easier to treat with AZA as they lack a competing metabolic pathway. In addition, these patients may develop fewer gastrointestinal side effects such as hepatotoxicity because high TPMT activity can lead to accumulation of 6-methylmercaptopurine with a consequent increased risk of developing hepatotoxicity.[13] If, on the contrary, methylated metabolites were essential for the therapeutic action of thiopurines in IBD disease, no therapeutic effect should have been observed in our TPMT deficient patients.

The precise mechanisms of thiopurine action in lymphocytes are still a matter of debate. Incorporation of 6-TGN into DNA may trigger cell apoptosis and seems to be essential for cytotoxicity in leukaemia derived cell lines. However, it has been shown that TPMT methylates 6-thioinosine-5′-monophosphate (TIMP), yielding the S-methylated derivative (methylTIMP) which is at least three times more potent an inhibitor of de novo purine synthesis than 6-TGN. This represents an alternative mechanism for cytotoxicity.[16] In order to achieve a particular therapeutically active percentage of 6-TGN incorporation into DNA, higher 6-TGN levels should be necessary in the case of TPMT deficiency which itself causes absence of methylTIMP. Despite a lower AZA dosage, it is therefore not surprising that minimal effective 6-TGN levels in TPMT deficient patients are higher than in TPMT wild-type patients.

Furthermore, although investigations with human leukaemic cells overexpressing TPMT have recently shown that methylmercaptopurine nucleotides decrease leukaemia cell viability independent of thiopurine incorporation into cellular DNA,[16] our report demonstrates that this mechanism is not essential for therapeutic efficacy in TPMT deficient patients with Crohn's disease and AZA therapy.

Reports of a ninefold increased risk of secondary malignancy in TPMT heterozygous children treated with thiopurines for remission maintenance in ALL[17] is a major concern. This risk was shown to be associated with very high 6-TGN levels which contribute to much increased genomic thioguanine incorporation. This risk is most probably conditional on the simultaneous exposure to other cytotoxic drugs (for example, topoisomerase-2 inhibitors or alkylating agents) and may therefore not apply to IBD patients. None the less, as a precaution, repeated determinations of 6-TGN levels and subsequent titration to the lowest effective dose should be routinely performed when TPMT deficient patients are treated with thiopurines.

Our observations should also be applicable to the recently described use of 6-thioguanine in IBD.[18 19]

We propose to investigate whether the introduction of a clinically effective and specific TPMT inhibitor could increase the overall response rate to thiopurine treatment while reducing toxicity.

It is to be hoped that nature's knocking out of a seemingly functionless enzyme may lead us towards a more individually tailored optimisation of drug treatment.

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