Journal of Crohn's and Colitis (2012) 6, 95–101

Available online at www.sciencedirect.com

SciVerse ScienceDirect

Hepatotoxicity associated with 6-methyl mercaptopurine formation during azathioprine and 6-mercaptopurine therapy does not occur on the short-term during 6-thioguanine therapy in IBD treatment

Dirk P. van Asseldonk*, Margien L. Seinen, Nanne K.H. de Boer, Ad A. van Bodegraven, Chris J. Mulder

Department of Gastroenterology and Hepatology, VU University Medical Center, Amsterdam, The Netherlands

Received 29 April 2011; received in revised form 1 July 2011; accepted 15 July 2011

KEYWORDS
6-Thioguanine;
Inflammatory bowel disease;
Hepatotoxicity;
Azathioprine;
6-Mercaptopurine;
6-Methyl mercaptopurine;

Abstract

Background and aims: High concentrations of methylated thiopurine metabolites, such as 6-methyl mercaptopurine, are associated with hepatotoxicity during administration of the conventional thiopurines azathioprine or 6-mercaptopurine in IBD patients. Metabolization of the non-conventional thiopurine 6-thioguanine does not generate 6-methyl mercaptopurine. Hence, the aim of our study was to evaluate hepatotoxicity during 6-thioguanine in IBD patients who previously failed conventional thiopurines due to 6-methyl mercaptopurine associated hepatotoxicity.
Methods: A retrospective single center intercept cohort study was performed of IBD patients using 6-thioguanine between January 2006 and July 2010 after failing conventional thiopurine therapy due to 6-methyl mercaptopurine associated hepatotoxicity. The primary outcome was the occurrence of 6-thioguanine induced hepatotoxicity, scaled according to the Common Terminology Criteria for Adverse Events.

Abbreviations 6-MMP, 6-methyl mercaptopurine; AZA, azathioprine; 6-MP, 6-mercaptopurine; 6-TG, 6-thioguanine; 6-TGN, 6-thioguanine nucleotides; TPMT, thiopurine S-methyl transferase; HBI, Harvey–Bradshaw Index; MTLWI, Modified Truelove and Witts Index.

* Corresponding author at: Gastroenterology and Hepatology, VU University Medical Centre, P.O. Box 7057, 1007 MB, Amsterdam, The Netherlands. Tel.: +31 204440613; fax: +31 204440554.
E-mail addresses: d.vanasseldonk@vumc.nl (D.P. van Asseldonk), ml.seinen@vumc.nl (M.L. Seinen), khn.deboer@vumc.nl (N.K.H. de Boer), v.bodegraven@vumc.nl (A.A. van Bodegraven), cjmulder@vumc.nl (C.J. Mulder).

1873-9946/$ - see front matter
doi:10.1016/j.crohns.2011.07.009

Results: Nineteen patients were included. Median duration of 6-thioguanine therapy (median daily dosage 21 mg (9–24)) was 23 weeks (6–96). Hepatotoxicity did not reoccur in 15 out of 19, whereas grade 1 toxicity persisted in 4 patients ($p<0.001$). Median aspartate aminotransferase and alanine aminotransferase concentrations decreased from 34 U/l (20–59) and 64 U/l (15–175) to 23 U/l (18–40; $p=0.003$) and 20 U/l (14–48; $p=0.019$), respectively.
Conclusion: Hepatotoxicity does not reoccur during 6-thioguanine treatment in most IBD patients who failed conventional thiopurines due to 6-methyl mercaptopurine associated hepatotoxicity. Hence, at least at short-term, 6-thioguanine appears a justifiable alternative thiopurine for these IBD patients.

1. Introduction

Drug therapy for inflammatory bowel disease (IBD) roughly consists of a remission induction phase and remission maintenance phase. The conventional thiopurines azathioprine (AZA) and 6-mercaptopurine (6-MP) have been used as maintenance treatment with reasonable success. However, on the long-term there is a fairly large proportion of IBD patients, up to 30 to 50%, which fails these therapies, mainly due to adverse events and therapy refractoriness.[1] A substantial part of treatment failures is believed to result from a deviant thiopurine metabolism. To become efficacious AZA and 6-MP need to be metabolized into the pharmacologically active 6-thioguanine nucleotides (6-TGN). However, catabolic conversions may hamper this bioactivation (Fig. 1). Thiopurine S-methyl transferase (TPMT), the activity of which follows a trimodal distribution in the Caucasian population, drives the methylation of AZA and 6-MP into 6-methyl mercaptopurine and 6-methyl mercaptopurine ribonucleotides, together abbreviated as 6-MMP.[2] High concentrations of 6-MMP are associated with overall treatment failure and hepatotoxicity in particular.[1,3] Despite dose escalation a proportion of patients fails to achieve therapeutic concentrations of 6-TGN (>235 pmol/8×10*8 red blood cells (RBC)), mostly in favor of 6-MMP production. These patients are called preferential 6-MMP metabolizers.[4,5] For this specific sub-group of patients alternative maintenance treatments have been suggested, including low dosed AZA or 6-MP in combination with allopurinol, methotrexate (presumably only in case of Crohn's disease) or anti-TNFα antibody therapies.[6,7] In addition, 6-thioguanine (6-TG) has been advocated as an escape/rescue maintenance treatment in patients who previously failed conventional thiopurine therapy.[8,9] Since 6-TG only needs one enzymatic conversion into 6-TGN and its metabolization does not result in 6-MMP production, we hypothesized 6-TG to be a convenient alternative thiopurine, in particular for preferential 6-MMP metabolizers. Hence, the main objective of this study was to explore 6-TG toxicity in preferential 6-MMP metabolizers with hepatotoxicity.

2. Materials and methods

2.1. Patient population

In accordance with the 2008 Declaration of Helsinki a retrospective single center intercept cohort study of IBD patients using 6-TG between January 2006 and July 2010 was performed. Diagnoses of Crohn's disease (CD) and ulcerative colitis (UC) were established by standard clinical, endoscopical and/or histological criteria.[10] All patients required immunosuppressive therapy (chronic active disease or glucocorticoid dependent) and previously failed AZA or 6-MP therapy due to

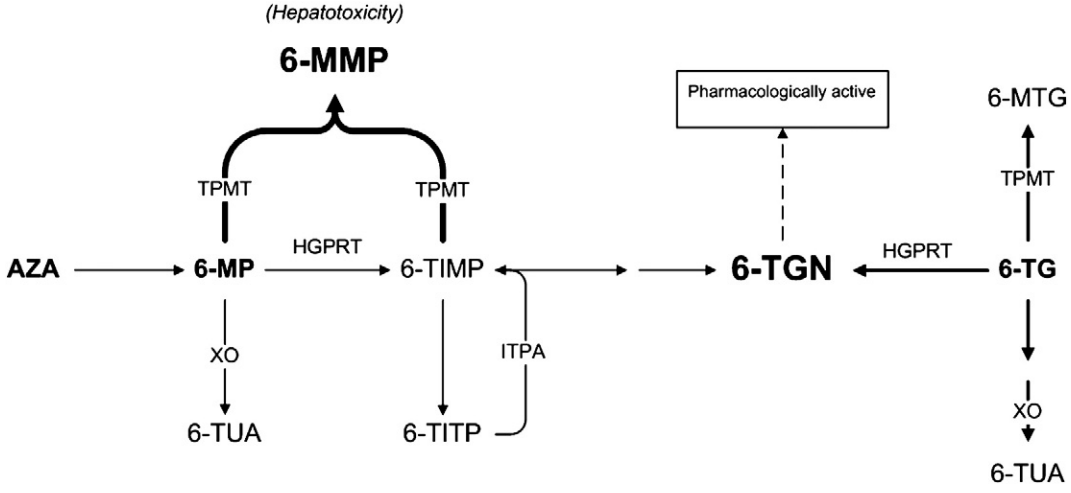

Figure 1 Thiopurine metabolism. Thiopurine metabolism in a preferential 6-methyl mercaptopurine (6-MMP) metabolizer results in the formation of high concentrations of 6-MMP at the cost of the production of pharmacologically active 6-thioguanine nucleotides (6-TGN). AZA, azathioprine; 6-MP, 6-mercaptopurine; 6-TUA, 6-thiouric acid; 6-TIMP, 6-thioinosine monophosphate; 6-TITP,6-thioinosine triphosphate; 6-TG, 6-thioguanine; 6-MTG, 6-methyl thioguanine; TPMT, thiopurine S-methyl transferase; HGPRT, hypoxanthine-guanine phosphoribosyl transferase; XO, xanthine oxidase; ITPA, inosine triphosphate pyrophosphohydrolase.

either adverse events or therapy resistance, according to the international guideline/recommendation.[11] Resistance to AZA or MP was defined as persisting or deteriorating active disease at 6 months of therapy despite dose escalation, if appropriate and feasible. 6-Thioguanine, prescribed in a daily dose of approximately 0.3 mg/kg, was administered as 18, 21 or 24 mg capsules (generic). Informed consent was obtained from all patients prior to initiation of 6-TG. Contraindications for receiving 6-TG were: active infection, (expected) pregnancy, lactation and pre-existing known liver disease.

Patients who had a preferential 6-MMP metabolism and developed hepatotoxicity during AZA or 6-MP therapy were identified and retrieved from a prospectively maintained cohort database. A preferential 6-MMP metabolism was arbitrarily defined as a 6-MMP/6-TGN ratio above 20 in combination with 6-MMP concentrations above 5700 pmol/8×10*8 RBC. Hepatotoxicity was defined as at least one of the following parameters over the upper limit of its normal range: aspartate aminotransferase>45 U/l, alanine aminotransferase>45 U/l, alkaline phosphatase>125 U/l, gamma glutamyl transferase>45 U/l and bilirubin>17 μmol/l. Patients with pre-existing liver test abnormalities, prior to initiation of the conventional thiopurines, were excluded from the analysis. Moreover, patients with taking serological blood tests suggestive of viral or auto-immune hepatitis as well as primary liver disease or liver injury possibly related to other medications were excluded from analysis.

Data concerning patient demographics, clinical characteristics, laboratory results, and co-treatment with 5-aminosalicylates, corticosteroids, and other immunomodulating drugs were collected from patient record files. An evaluation point was designated as a clinic visit, usually occurring every 3 months, corresponding to each thiopurine metabolite measurement that was obtained along with routine blood tests: one prior to switching to 6-TG after at least 6 weeks of AZA or 6-MP and the other after at least 6 weeks of 6-TG treatment.

2.2. Outcome measures

The primary outcome was the frequency and severity of hepatotoxicity during 6-TG treatment compared with during conventional thiopurine therapies. Secondary outcomes included other adverse events, thiopurine metabolite measurement, disease activity and corticosteroid use. The severity of hepatotoxicity and the other adverse events was scaled according to the Common Terminology Criteria for Adverse Events (CTCAE, version 4.03) (Table 2).[12] All outcome measures were assessed at both clinical evaluation points. Corticosteroid use was defined as the use of an equivalent of prednisolone 10 mg/day or budesonide 3 mg/day during both thiopurine regimens. Thiopurine metabolite concentrations were determined in RBC from heparinized blood (6 mL) by reversed-phase high-performance liquid chromatography according to a modified method of Dervieux and Boulieu as previously reported.[13,14] The obtained 6-TGN values were subsequently adjusted to values that would have been obtained by using the method of Lennard et al.[15,16] Disease activity was assessed by calculating the Harvey–Bradshaw Index (HBI) for CD and the Modified Truelove and Witts Index (MTLWI) for UC, if data were available.[17,18] Clinical response was defined by a HBI score reduction from baseline of ≥50% or a score <5 for CD and a MTLWI score reduction from baseline of ≥50% or a score of <3 for UC.

2.3. Statistical methods

Quantitative variables were described as means with their standard deviations or as medians with their range if not normally distributed. Depending on the distribution, parametric and non-parametric tests including *T*-test, Wilcoxon signed rank test and Sign exact test, were used to test for differences within and between groups. P values less than 0.05 were considered statistically significant. SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

3. Results

3.1. Patient characteristics

Nineteen patients including five males were retrieved from the prospectively maintained IBD database and eligible for analysis. Their mean age was 44 years (SD 11). Out of the 19 included patients, 12 were diagnosed with ulcerative colitis and 7 had Crohn's disease. Patient and disease characteristics are tabulated in Table 1. Thiopurine pre-treatment included AZA (n=5), 6-MP (n=9), or both (n=5). Median duration of pre-treatment was 9 months, ranging from 0 to 104 months. Pre-treatment metabolite concentrations were fitting the criteria of a preferential 6-MMP metabolism with a median 6-MMP/6-TGN ratio of 126 (42–323), a median 6-MMP concentration of 18,150 pmol/8×10^8 RBC (6280–33,570) and a median 6-TGN concentration of 127 pmol/8×10^8 RBC (35–280). All patients developed hepatotoxicity during thiopurine pre-treatment; 4 patients showed grade 2, whereas 15 showed grade 1 toxicity. Other observed adverse events during preceding thiopurine treatment included myelotoxicity (n=3), nausea (n=2) and arthralgia (n=1).

3.2. 6-Thioguanine therapy

With a median duration of 23 weeks (range 6–96), 6-TG was prescribed in a mean dose of 21 mg per day (SD 4 mg), corresponding to 0.30 mg/kg (SD 0.05). Three out of 19 patients (16%) developed adverse events during 6-TG therapy, none of which resulted in drug withdrawal or dose adjustment. Two out of these three had developed myelotoxicity in combination with hepatotoxicity during preceding thiopurine treatment, one of whom experienced arthralgia grade 1 and the other a *Clostridium difficile* enterocolitis grade 2 during 6-TG therapy. The third patient developed myalgia grade 1. During follow-up the median 6-TGN concentration was 427 pmol/8×10^8 RBC ranging from 126 to 800 pmol/8×10^8 RBC. As expected no 6-MMP was detected. Additional laboratory results are shown in Table 3.

3.3. 6-Thioguanine induced hepatotoxicity

During 6-TG treatment the frequency of hepatotoxicity, indicated by the CTCAE scores, decreased as compared during preceding thiopurine treatment ($p<0.001$). In Fig. 2 it is depicted that median AST and ALT concentrations

Table 1 Patient characteristics (n = 19).

Gender (M:F)		5:14
Age at diagnosis IBD, year (S.D.)		32.2 (11.9)
Body mass index (S.D.)		24.1 (3.7)
Disease classification and localization		
CD		7
Below 17 year	(A1)	–
17–40 year	(A2)	3
Above 40 year	(A3)	4
Ileal	(L1)	2
Colonic	(L2)	1
Ileocolonic	(L3)	4
Isolated upper disease	(L4)	–
Non-stricturing, non-penetrating	(B1)	1
Stricturing	(B2)	5
Penetrating	(B3)	1
Perianal disease modifier	(p)	2
UC		12
Proctitis	(E1)	2
Left-sided	(E2)	8
Pancolitis	(E3)	2
Duration disease to start 6-TG, year (range)		8.8 (0.5–32.9)
Pre-treatment (AZA:6-MP:both)		5:9:5
AZA dose in mg/day (S.D.)		145 (37)
AZA dose in mg/kg/day (S.D.)		2.26 (0.48)
6-MP dose in mg/day (S.D.)		96 (22)
6-MP dose in mg/kg/day (S.D.)		1.4 (0.29)
Duration pre-treatment, months (range)		9 (1–104)
Metabolite concentrations with AZA/6-MP		
6-MMP, pmol/8×10^8 RBC (range)		18,150 (6280–33,570)
6-TGN, pmol/8×10^8 RBC (range)		127 (35–280)
6-MMP/6-TGN ratio (range)		126 (42–323)
Age at initiation 6-TG, year (S.D.)		44.3 (11.2)
Days between pre-treatment and 6-TG (range)		9 (0–494)

decreased from 34 U/l (20–59) and 64 U/l (15–175) to 23 U/l (18–40; p = 0.003) and 20 U/l (14–48; p = 0.019). Hepatotoxicity completely resolved In 15 patients (79%): 4 from grade 2 toxicity to grade 0, and 11 from grade 1 to 0 (Fig. 3). In another 4 patients hepatotoxicity remained grade 1, albeit very subtle with ALT concentrations of 46, 47 and 48 U/l in three and a bilirubine concentration of 22 μmol/l in one. Two out of these four patients also experienced other adverse events during 6-TG (one arthralgia and the other myalgia). Total 6-TG dosage, 6-TG dosage per kg, 6-TGN concentration

Table 2 Common Terminology Criteria for Adverse Events (CTCAE v4.0)(12).

Grade				
Adverse event	1	2	3	4
AST increased	>ULN–3.0×ULN	>3.0–5.0×ULN	>5.0–20.0×ULN	>20.0×ULN
ALT increased	>ULN–3.0×ULN	>3.0–5.0×ULN	>5.0–20.0×ULN	>20.0×ULN
AP increased	>ULN–2.5×ULN	>2.5–5.0×ULN	>5.0–20.0×ULN	>20.0×ULN
Gamma-GT increased	>ULN–2.5×ULN	>2.5–5.0×ULN	>5.0–20.0×ULN	>20.0×ULN
Bilirubine increased	>ULN–1.5×ULN	>1.5–3.0×ULN	>3.0–10.0×ULN	>10.0×ULN
Arthralgia	Mild pain	Moderate pain	Severe pain	–
Myalgia	Mild pain	Moderate pain	Severe pain	–
Enterocolitis	–	>3 unformed stools per 24 h or duration of illness >48 h; moderate abdominal pain	Antibiotic, endoscopic or operative intervention indicated; profuse watery diarrhea; bloody stools; fever	Life-threatening consequences; urgent intervention indicated

The upper limits of normal (ULN): AST, aspartate aminotransferase 45 U/l; ALT, alanine aminotransferase 45 U/l; AP, alkaline phosphatase 125 U/l; Gamma-GT, gamma glutamyl transferase 45 U/l; bilirubine 17 μmol/l.

Table 3 Laboratory results.

Parameter	AZA/6-MP	6-TG	P
AST (U/l)	34 (20–59)	23 (18–40)	0.003
ALT (U/l)	64 (15–175)	20 (14–48)	0.019
AP (U/l)	67 (35–114)	74 (32–112)	0.649
Gamma-GT (U/l)	28 (9–116)	21 (6–45)	0.040
Bilirubine (mmol/l)	13 (5–31)	8 (6–22)	0.030
Hemoblobine (mmol/l)	7.7 (4.3–9.0)	8.2 (5.9–9.2)	0.143
Leukocyte count (×10*9/l)	6.4 (1.0–9.8)	6.5 (3.9–15.3)	0.210
Platelet count (×10*9/l)	297 (35–518)	331 (129–422)	0.935
C reactive protein (mg/l)	2 (2–255)	3 (2–83)	0.84

AZA/6-MP = azathioprine and or 6-mercaptopurine pre-treatment; median duration 9 months (1–104).
6-TG = 6-thioguanine therapy; median duration 23 weeks (6–96).

and disease activity was not different in these 4 patients compared to the other 11 (data not shown). All patients continued 6-TG therapy as these adverse events were all considered of minor clinical importance in comparison to the beneficial therapeutical effects of 6-TG treatment. In two patients who had been using 6-TG for at least 1 year a routine liver biopsy was performed that in both cases revealed no histological abnormalities. None of these two patients were among the four patients with persisting or recurrence of hepatotoxicity.

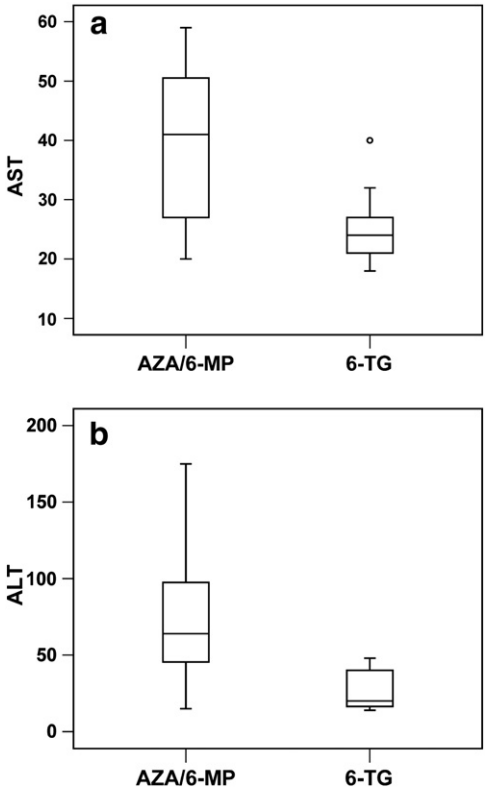

Figure 2 Changes in transaminase concentrations. (a) AST decreases from a median of 34 U/l (20–59) during preceding thiopurine treatment to 23 U/l (18–40) during 6-TG therapy; p=0.003. (b) ALT decreases from a median of 64 U/l (15–175) to 20 U/l (14–48); p=0.019.

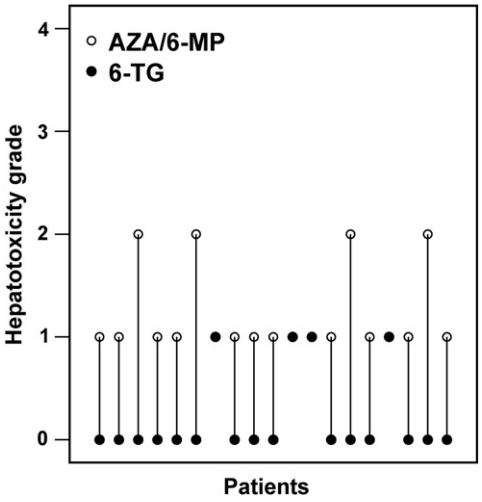

Figure 3 Severity of hepatotoxicity. Severity of hepatotoxicity during preceding thiopurine treatment (open dots) and 6-TG therapy (filled dots), assessed by the Common Terminology Criteria for Adverse Events; p=<0.001. In 4 patients the hepatotoxicity grade was equal between both treatments.

3.4. Disease activity

From 10 out of the 12 UC patients we were able to assess the MTLWI both during pre-treatment and during 6-TG treatment. Five out of 10 showed a clinical response during 6-TG therapy. In 4 out of the 5 patients not showing a clinical response, MTLWI did not change between both treatments and remained equal or above 3 points. Deterioration of disease activity during 6-TG therapy occurred in one patient. From 5 out of 7 CD patients we were able to assess HBI during both types of thiopurine treatment. In all 5 patients disease activity did not change and remained below 5, thus showing a persistent/on-going clinical response.

3.5. Concomitant treatment

During preceding thiopurine treatment seven patients (six UC and one CD) received 5-aminosalicylates, while eight (seven UC and one CD) received these drugs during 6-TG therapy. Six patients needed corticosteroids during pre-treatment; these drugs could be completely tapered in three patients during 6-TG therapy. Three patients remained corticosteroid dependent but dosages could be reduced, while another four needed a corticosteroid course during 6-TG therapy. One patient received adalimumab in addition to 6-TG.

4. Discussion

This study describes a series of IBD patients in most of whom thiopurine associated hepatotoxicity did not reoccur during short-term 6-TG administration. Liver test abnormalities

were statistically significantly ameliorated during 6-TG administration. Moreover, of the 19 patients with 6-MMP associated hepatotoxicity, 15 did not show any signs of hepatotoxicity with 6-TG. In the remaining four patients liver test abnormalities were subtle and seemed not to deteriorate with 6-TG. Only three patients developed some other minor adverse events that did not result in 6-TG withdrawal, suggesting 6-TG to be well-tolerated in this difficult to treat group of patients. These results are similar to the tolerability reported in previous literature.[9,19] In addition, 6-TG seemed to be effective for remission maintenance in most patients, although few patients needed on-going corticosteroid therapy.

During conventional thiopurine therapy hepatotoxicity may occur in up to 10% and is a major determinant for therapeutic failure.[20] Thiopurine associated hepatotoxicity may be either an idiosyncratic reaction or a dose-dependent phenomenon, and has been associated with high concentrations of 6-MMP (>5700 pmol/8×10^8 RBC).[4] Our study further corroborates this association since 6-TG metabolism does not bear 6-MMP and hepatotoxicity generally does not reoccur with 6-TG. The pathogenesis of thiopurine associated hepatotoxicity is not known, although animal models have shown deprivation of intracellular reduced glutathion concentrations that predisposes to oxidative stress, and antioxidative treatment strategies might be beneficial.[21] It remains indistinct why four patients developed hepatotoxicity during 6-TG therapy. Since two of these four patients were part of the three patients who also experienced other adverse events during 6-TG therapy, a common cause might be suspected. These patients may exhibit an aberrant 6-TG metabolism, such as altered TPMT or hypoxanthine guanine phosphoribosyl transferase (HGPRT) activities. However, this could not be endorsed by 6-TGN concentrations. As absolute concentrations of the liver test abnormalities were only just above the upper limit of their normal range, their clinical significance might be overestimated. Although unlikely, the presence of liver disease secondary to IBD such as primary sclerosing cholangitis could not be excluded in this study.

Safety concerns about the use of 6-TG have emerged over the past years. This seems to be a dose-dependent effect rather than solely a drug specific effect.[22] While liver biopsies in patients treated with a high dose of 6-TG often reveals pathohistological abnormalities including nodular regenerative hyperplasia, this is not the case with the use of lower dosages.[23–25] Although we believe that 6-TG has a relatively favorable benefit–risk ratio if dosed around 0.3 mg/kg daily, we still recommend to assess serum liver tests, blood cell counts and perform liver biopsies on a regular basis in line with international recommendations as part of drug safety monitoring.[11] The duration of follow-up of our study was relatively short, therefore only two patients had underwent a liver biopsy so far. A preferential 6-MMP metabolism upon conventional thiopurine therapy is not only related with hepatotoxicity, but also with other adverse event and drug resistance.[26] 6-Thioguanine might be indicated in all patients with thiopurine treatment failure associated with a preferential 6-MMP metabolism, since dose escalation of conventional thiopurines will probably result in higher concentrations of 6-MMP, while 6-TGN concentrations remain relatively low. Interestingly, in our study the range of pretreatment duration was strikingly large. This implies that there may be interindividual differences in thiopurine metabolism, which might change over time into a skewed metabolism, and differences in susceptibility to hepatotoxicity.

As a proportion of patients in this study was corticosteroid dependent during 6-TG therapy, it is of interest to evaluate whether dose escalation would increase 6-TG efficacy or whether these specific patients represent a group of primary thiopurine non-responders, including non-response to 6-TG. Alternatively, the administration of the xanthine oxidase inhibitor allopurinol in addition to a low dose of AZA or 6-MP might be a successful option, in particular in preferential 6-MMP metabolizers but presumably also in other cases of thiopurine treatment failure.[27,28] However, safety concerns regarding the occurrence of harmful myelotoxicity exist, while any liver biopsy data to show safety with regard to NRH-induction are lacking. In addition, there is relatively little clinical experience with this combination treatment in contrast to 6-TG therapy.

Although being a cohort study, our study does suffer from limitations most of which coincide with its retrospective design. Clinical evaluation points and washout periods were not standardized that makes comparability more difficult. In addition, this study was not designed nor powered to study or establish 6-TG efficacy. Although the number of participants is relatively small, as a proof of principle this study further substantiates the association between a skewed thiopurine metabolism and the occurrence of hepatotoxicity. There is a strong need for a prospectively designed controlled clinical trial in which 6-MP is compared with 6-TG with regard to both efficacy and safety. As a result of the simplistic metabolism of 6-TG with less potentially harmful metabolites, 6-TG might be better tolerated with comparable effectiveness provided that it is properly dosed. At present, we still recommend performing careful routine safety assessments upon its prescription.

5. Conclusion

Conventional thiopurine therapy is frequently withdrawn, hepatotoxicity often being the reason. A substantial part of this toxicity is associated with high concentrations of 6-MMP resulting from a deviant thiopurine metabolism. For these patients 6-TG might be an attractive alternative thiopurine, which generally does not induce such liver injury since its metabolization does not involve the production of 6-MMP.

Conflict of interest

None declared.

Acknowledgement

DPA contributed to the design of the study, collected and analyzed the data and wrote the draft of the paper. MLS has critically reviewed the paper. NKB has critically reviewed the paper. AAB has performed acquisition of data and critically reviewed the paper. CJM contributed to the conception and design of the study, performed acquisition of data and critically reviewed the paper. All authors have approved the final version of the manuscript before

submission. CJM received an unrestricted grant from HLW Pharma bv, Helmond, the Netherlands. HLW Pharma was not involved in any part of this study.

References

1. Jharap B, Seinen ML, de Boer NKH, van Ginkel JR, Linskens RK, Kneppelhout JC, Mulder CJ, Van Bodegraven AA. Thiopurine therapy in inflammatory bowel disease patients: analyses of two 8-year intercept cohorts. *Inflamm Bowel Dis* 2010;**16**:1541–9.
2. Schaeffeler E, Fischer C, Brockmeier D, Wernet D, Moerike K, Eichelbaum M, Zanger UM, Schwab M. Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. *Pharmacogenetics* 2004;**14**: 407–17.
3. Dubinsky MC, Lamothe S, Yang HY, Targan SR, Sinnett D, Theoret Y, Seidman EG. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology* 2000;**118**:705–13.
4. Dubinsky MC, Yang H, Hassard PV, Seidman EG, Kam LY, Abreu MT, Targan SR, Vasiliauskas EA. 6-MP metabolite profiles provide a biochemical explanation for 6-MP resistance in patients with inflammatory bowel disease. *Gastroenterology* 2002;**122**:904–15.
5. Gardiner SJ, Gearry RB, Burt MJ, Ding SL, Barclay ML. Severe hepatotoxicity with high 6-methylmercaptopurine nucleotide concentrations after thiopurine dose escalation due to low 6-thioguanine nucleotides. *Eur J Gastroenterol Hepatol* 2008;**20**: 1238–42.
6. Dignass A, Van Assche G, Lindsay JO, Lemann M, Soderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollon F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP. The second European evidence-based consensus on the diagnosis and management of Crohn's disease: current management. *J Crohns Colitis* 2010;**4**:28–62.
7. Sparrow MP, Hande SA, Friedman S, Cao D, Hanauer SB. Effect of allopurinol on clinical outcomes in inflammatory bowel disease nonresponders to azathioprine or 6-mercaptopurine. *Clin Gastroenterol Hepatol* 2007;**5**:209–14.
8. Herrlinger KR, Deibert P, Schwab M, Kreisel W, Fischer C, Fellermann K, Stange EF. Remission maintenance by tioguanine in chronic active Crohn's disease. *Aliment Pharmacol Ther* 2003;**17**:1459–64.
9. Van Asseldonk DP, Jharap B, Kuik DJ, De Boer NK, Westerveld BD, Russel MG, Kubben FJ, Van Bodegraven AA, Mulder CJ. Prolonged thioguanine therapy is well tolerated and safe in the treatment of ulcerative colitis. *Dig Liver Dis* 2011;**43**:110–5.
10. Lennard-Jones JE. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989;**170**:2–6.
11. De Boer NK, Reinisch W, Teml A, Van Bodegraven AA, Schwab M, Lukas M, Ochsenkuhn T, Petritsch W, Knoflach P, Almer S, Van der Merwe SW, Herrlinger KR, Seiderer J, Vogelsang H, Mulder CJ. 6-Thioguanine treatment in inflammatory bowel disease: a critical appraisal by a European 6-TG working party. *Digestion* 2006;**73**:25–31.
12. US Department of Health and Human Services, National Institute of Health, National Cancer institute. Common terminology criteria for adverse events v4.03. Accessed March 2011 at: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf. 2010.
13. Dervieux T, Boulieu R. Simultaneous determination of 6-thioguanine and methyl 6-mercaptopurine nucleotides of azathioprine in red blood cells by HPLC. *Clin Chem* 1998;**44**: 551–5.
14. De Graaf P, Vos RM, De Boer NH, Sinjewel A, Jharap B, Mulder CJ, Van Bodegraven AA, Veldkamp AI. Limited stability of thiopurine metabolites in blood samples: relevant in research and clinical practise. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010;**878**:1437–42.
15. Lennard L, Singleton HJ. High-performance liquid chromatographic assay of the methyl and nucleotide metabolites of 6-mercaptopurine: quantitation of red blood cell 6-thioguanine nucleotide, 6-thioinosinic acid and 6-methylmercaptopurine metabolites in a single sample. *J Chromatogr* 1992;**583**: 83–90.
16. Shipkova M, Armstrong VW, Wieland E, Oellerich M. Differences in nucleotide hydrolysis contribute to the differences between erythrocyte 6-thioguanine nucleotide concentrations determined by two widely used methods. *Clin Chem* 2003;**49**:260–8.
17. Harvey RF, Bradshaw MJ. Measuring Crohn's disease activity. *Lancet* 1980;**1**:1134–5.
18. Lichtiger S, Present DH. Preliminary report: cyclosporin in treatment of severe active ulcerative colitis. *Lancet* 1990;**336**: 16–9.
19. Bonaz B, Boitard J, Marteau P, Lemann M, Coffin B, Flourie B, Belaiche J, Cadiot G, Metman EH, Cortot A, Colombel JF. Tioguanine in patients with Crohn's disease intolerant or resistant to azathioprine/mercaptopurine. *Aliment Pharmacol Ther* 2003;**18**:401–8.
20. Bastida G, Nos P, Aguas M, Beltran B, Rubin A, Dasi F, Ponce J. Incidence, risk factors and clinical course of thiopurine-induced liver injury in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2005;**22**:775–82.
21. Menor C, Fernandez-Moreno MD, Fueyo JA, Escribano O, Olleros T, Arriaza E, Cara C, Lorusso M, Di paola M, Roman ID, Guijarro LG. Azathioprine acts upon rat hepatocyte mitochondria and stress-activated protein kinases leading to necrosis: protective role of N-acetyl-L-cysteine. *J Pharmacol Exp Ther* 2004;**311**: 668–76.
22. De Boer NK, Jharap B, Mulder C, Van Bodegraven AA. Low and adequately dosed 6-thioguanine: not so bad after all. *Inflamm Bowel Dis* 2008;**14**:1166–7.
23. Geller SA, Dubinsky MC, Poordad FF, Vasiliauskas EA, Cohen AH, Abreu MT, Tran T, Martin P, Vierling JM, Targan SR. Early hepatic nodular hyperplasia and submicroscopic fibrosis associated with 6-thioguanine therapy in inflammatory bowel disease. *Am J Surg Pathol* 2004;**28**:1204–11.
24. Gilissen LP, Derijks LJ, Driessen A, Bos LP, Hooymans PM, Stockbrugger RW, Engels LG. Toxicity of 6-thioguanine: no hepatotoxicity in a series of IBD patients treated with long-term, low dose 6-thioguanine. Some evidence for dose or metabolite level dependent effects? *Dig Liver Dis* 2007;**39**: 156–9.
25. Van Asseldonk DP, Jharap B, De Boer NH, Zondervan PE, Bloemena E, Den Hartog G, Westerveld BD, kolkman JJ, Engels LG, Van Bodegraven AA, Mulder C. Liver histology of IBD patients who are treated with 6-thioguanine due to failure of conventional thiopurines reveals very few cases of nodular regenerative hyperplasia. *Gastroenterology* 2010;**138**:S-62.
26. Hindorf U, Lindqvist M, Hildebrand H, Fagerberg U, Almer S. Adverse events leading to modification of therapy in a large cohort of patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2006;**24**:331–42.
27. Sparrow MP. Use of allopurinol to optimize thiopurine immunomodulator efficacy in inflammatory bowel disease. *Gastroenterol Hepatol* 2008;**4**:505–11.
28. Ansari A, Patel N, Sanderson J, O'Donohue J, Duley JA, Florin TH. Low-dose azathioprine or mercaptopurine in combination with allopurinol can bypass many adverse drug reactions in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2010;**31**:640–7.