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Allopurinol safely and effectively optimizes tioguanine metabolites in inflammatory bowel disease patients not responding to azathioprine and mercaptopurine

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SUMMARY

Background: Many non-responders to azathioprine or mercaptopurine (6-mercaptopurine) have high normal thiopurine methyltransferase activity and preferentially metabolize mercaptopurine to produce 6-methylmercaptopurine instead of the active 6-tioguanine (6-tioguanine) metabolites.

Aim: To describe the use of allopurinol in mercaptopurine/azathioprine non-responders to deliberately shunt metabolism of mercaptopurine towards 6-tioguanine.

Methods: Fifteen thiopurine non-responders whose metabolites demonstrated preferential metabolism towards 6-methylmercaptopurine are described. Subjects were commenced on allopurinol 100 mg po daily and mercaptopurine/azathioprine was reduced to 25–50% of the original dose. Patients were followed clinically and with serial 6-tioguanine and 6-methylmercaptopurine metabolite measurements.

Results: After initiating allopurinol, 6-tioguanine levels increased from a mean of 185.73 ± 17.7 to 385.4 ± 41.5 pmol/8×10^8 red blood cells ($P < 0.001$), while 6-methylmercaptopurine decreased from a mean of 10 380 ± 1245 to 1732 ± 502 pmol/8×10^8 RBCs ($P < 0.001$). Allopurinol led to a decrease in white blood cell from a mean of 8.28 ± 0.95 to 6.1 ± 0.82 $\times 10^8$/L ($P = 0.01$).

Conclusions: The addition of allopurinol to thiopurine non-responders with preferential shunting to 6-methylmercaptopurine metabolites appears to be an effective means to shift metabolism towards 6-tioguanine.

INTRODUCTION

In recent years, the thiopurine immunomodulators azathioprine (AZA) and mercaptopurine (6-mercaptopurine, MP) have become increasingly well established in the therapeutic armamentarium of inflammatory bowel disease (IBD), although the evidence supporting their use is stronger for Crohn's disease (CD) than ulcerative colitis (UC). In CD, there is controlled trial evidence to support the use of AZA/MP as induction agents in mild/moderate inflammatory CD, fistulizing CD, as a steroid-sparing agent in steroid-dependent CD and for the maintenance of remission.[1, 2] A meta-analysis of nine randomized, placebo-controlled trials of AZA or MP as induction or maintenance agents in CD was published in 1995 and revealed an odds ratio (OR) of response favouring thiopurines compared with placebo of 3.09 (95%CI: 2.45–3.91) in active CD and 2.27 (95% CI: 1.76–2.93) in quiescent disease. A steroid-sparing effect was seen in both active disease (OR: 3.69) and quiescent

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disease (OR: 4.64).[3] By comparison, in UC, controlled trials suggest efficacy of thiopurines only as steroid-sparing induction agents in steroid-dependent UC and as maintenance agents with possible benefits in chronic active steroid-refractory disease.[4]

In both CD and UC at least 50% of patients do not respond to recommended doses despite an adequate duration of therapy with the thiopurine immunomodulators MP and AZA. Most recently, lack of response has been attributed to differences in individual variations in drug metabolism.[5, 6] Mercaptopurine and AZA are both inactive prodrugs and are metabolized via three main enzymatic pathways to produce the nucleotide metabolites 6-tioguanine (6-tioguanine, 6-TGN), 6-methylmercaptopurine (6-MMP) and 6-thiouracil (Figure 1). In 2000, Dubinsky *et al.*[6] reported that 6-TGN is the active therapeutic metabolite by correlating serum 6-TGN levels with both clinical response and the risk of myelotoxicity. Three tioguanine nucleotides [6-tioguanine monophosphate (6-TGMP), diphosphate (6-TGDP) and triphosphate (6-TGTP)] are recognized and distinguished by the number of phosphate residues attached during the anabolic conversion of 6-thiosine 5′-monophosphate. Previously, it was believed that 6-TGN exerted its immunosuppressive action solely through incorporation into cellular nucleic acids, thereby inhibiting lymphocyte proliferation. However, it has recently been shown that 6-TGTP may also actively stimulate lymphocytic apoptosis by inhibiting RacI (an intracellular enzyme involved in nuclear factor-κB activation) in T lymphocytes.[7] In contrast to the therapeutic potential of the 6-TGNs, serum 6-MMP levels correlated with hepatotoxicity (elevated serum transaminases). In the initial retrospective series the frequency of therapeutic response increased at 6-TGN levels >235 pmol/8×10^8 erythrocytes (OR: 5.0) and transaminase elevation correlated with 6-MMP levels >5700 pmol/8×10^8 erythrocytes.[6] Their study also demonstrated that 6-TGN concentrations were inversely correlated with genetically determined functional activity of the thiopurine methyltransferase (TPMT) enzyme while elevations in 6-MMP were associated with high, functional TPMT activity.

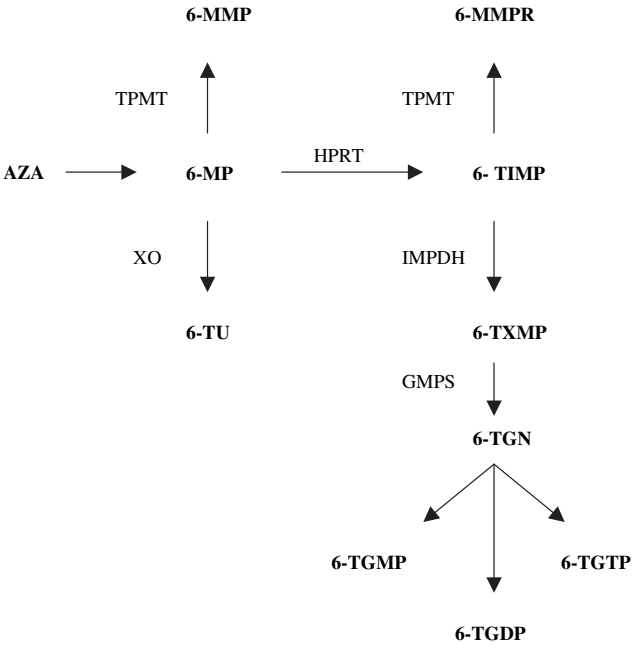

Figure 1. Metabolism of mercaptopurine (XO, xanthine oxidase; TPMT, thiopurine methyltransferase; HPRT, hypoxanthine phosphoribosyltransferase; 6-TIMP, 6-thiosine 5′-monophosphate; IMPDH, inosine monophosphate dehydrogenase; GMPS, guanosine monophosphate synthetase; 6-TGMP, 6-tioguanine monophosphate; 6-TGDP, 6-tioguanine diphosphate; 6-TGTP, 6-tioguanine triphosphate).

Subsequently, Dubinsky *et al.*[8] attempted to attain 'therapeutic levels' in 51 IBD patients defined as clinical non-responders to AZA/MP with dose escalation, however only 14 of 51 patients (27%) responded. Median 6-TGN levels rose 72% in responders, and only 20% in non-responders ($P = 0.002$). Conversely, non-responders were characterized by a preferential rise in 6-MMP, suggesting that the dominant metabolic pathways are different among responders and non-responders. However, other studies have reported conflicting results regarding the usefulness of metabolite measurements to optimize thiopurine dosing and predict the risk of leucopoenia in IBD. Lowry *et al.* reported that median 6-TGN concentrations were similar in 56 patients with active disease (6-TGN: 139 pmol/8×10^8 erythrocytes) and 114 patients in remission (6-TGN: 131 pmol/8×10^8 erythrocytes, $P = 0.26$), and that there was no correlation between 6-TGN concentrations and leucocyte counts.[9] A prospective, randomized trial comparing using metabolite measurements to using weight-based dosing for thiopurine dose optimization is currently underway and will hopefully help clarify some of these controversies.

These important revelations regarding thiopurine metabolism would make inhibition of TPMT a logical target to 'optimize' thiopurine metabolite levels, as population studies have revealed that the distribution of TPMT activity is trimodal: 0.3% of individuals have low activity, 11% have intermediate activity and 89% have normal, or

high, activity.[10] Xanthine oxidase (XO) is also involved early in the metabolic pathway of both MP as well as the 6-TGNs via oxidation to the inactive metabolite 6-thiouric acid (6-TU). In an attempt to modulate the metabolism of AZA we have introduced low doses of allopurinol, a competitive inhibitor of XO, to improve the purine metabolic profiles in non-responding IBD patients with documented disproportionate 6-MMP:6-TGN ratios.

MATERIALS AND METHODS

Patient population

Fifteen predominantly steroid-dependent out-patients from two tertiary referral IBD clinics who were non-responders to MP or AZA with 6-TGN levels <230 pmol/8×10^8 erythrocytes and 6-MMP levels >5000 pmol/8×10^8 erythrocytes are described. Nine patients had CD, four had UC and two had indeterminate colitis (IC). TMPT activity had been previously measured in seven of the 15 patients and was normal in all cases. Six of the 15 patients were also receiving aminosalicylates. All patients were treated with allopurinol at a dose of 100 mg orally daily and the dose of thiopurine was reduced, initially or subsequently, to 25–50% of the original dose. Complete blood counts were obtained on a weekly basis for the first month of therapy and MP metabolites were measured by Prometheus Laboratories (San Diego, CA, USA) approximately 2–4 weeks after initiating allopurinol. Prior to starting allopurinol, four patients were receiving AZA at a mean dose of 188 mg daily (mean 2.0 mg/kg/day, range: 50–250 mg) while 11 patients were receiving MP at mean daily doses of 92 mg (mean 1.2 mg/kg/day, range: 75–125 mg). After commencement of allopurinol, the dose of AZA was reduced to a mean dose of 88 mg daily (mean 0.9 mg/kg/day, range: 50–100 mg) and MP was reduced to a mean daily dose of 51 mg (mean 0.7 mg/kg/day, range: 25–100 mg).

Outcome measures and statistical analysis

To analyse the effects of allopurinol on thiopurine metabolism, corresponding values of 6-TGN and 6-MMP levels (pmol/8×10^8 red blood cells) and white blood cell counts (WBC; $\times 10^8$/L), before and after commencing allopurinol, were recorded and compared using the non-parametric Wilcoxon signed rank test. When 6-TGN and 6-MMP levels were not detected by the assay, the lower limits of detection of 9 pmol/8×10^8 RBCs and 294 pmol/8×10^8 RBCs respectively were used in the statistical calculations. Statistical analysis was performed using STATA SE 8.0.

RESULTS

After allopurinol was started, 6-TGN levels increased significantly from a mean of 185.7 ± 17.7 (S.E.) to 385.4 ± 41.5 pmol/8×10^8 RBCs ($P < 0.001$; Figure 2), while 6-MMP decreased significantly from a mean of 10 380 ± 1245 to 1732 ± 502 pmol/8×10^8 RBCs ($P < 0.001$; Figure 3). The addition of allopurinol led to a significant decrease in WBC from 8.28 ± 0.95 to 6.1 ± 0.82 $\times 10^8$/L ($P = 0.01$; Figure 4). Five patients developed leucopoenia (defined as WBC < 3.5×10^8/L) that resolved with thiopurine dose reduction. One additional patient developed rapid leucopoenia with a WBC count of 1.1×10^8/L and allopurinol was discontinued immediately. This patient did not have additional metabolites measured and therefore was not included in the statistical analysis.

Although formal disease activity indices were not performed the clinical impression was that most patients improved. Corticosteroids could be ceased in four of the 15 patients after the addition of allopurinol. However, a total of three patients subsequently required escalation of therapy to antitumour necrosis factor (TNF)-*alpha* agents. A further patient relapsed 2 months after allopurinol was added, with initial response, and required surgery. The response to addition of allopurinol was seen equally in all patient groups, regardless of the individual diagnosis of IBD, be it CD, UC, or IC. The response was also equally seen in

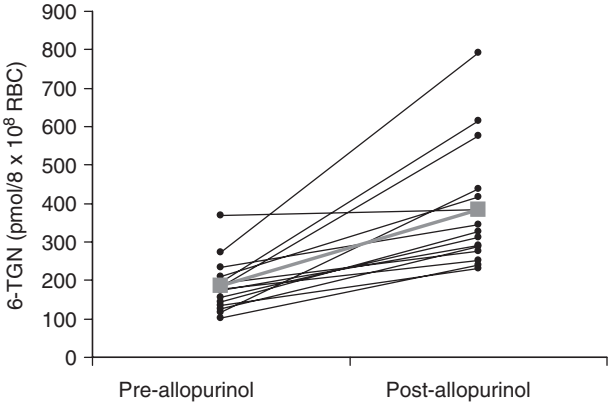

Figure 2. 6-Tioguanine (6-TGN; pmol/8×10^8 RBC) levels before and after addition of allopurinol. Thin lines show each individual patient and thick line shows mean value.

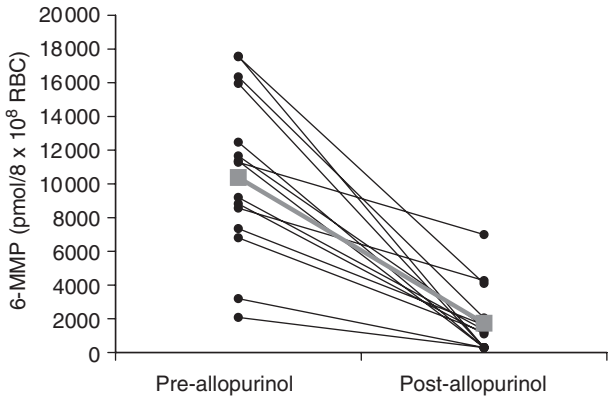

Figure 3. 6-Methylmercaptopurine (6-MMP; pmol/8 × 10^8 RBC) levels before and after addition of allopurinol. Thin lines show each individual patient and thick line shows mean value.

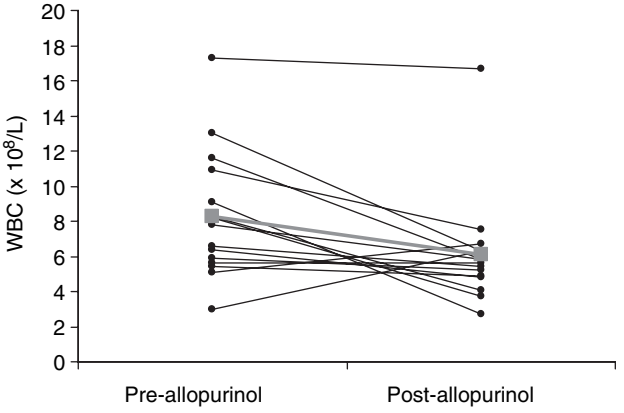

Figure 4. White blood cell count (WBC; ×10^8/L) before and after addition of allopurinol. Thin lines show each individual patient and thick line shows mean value.

patients taking and not taking concurrent aminosalicylates. Table 1 outlines the biochemical response to potentiation of thiopurine immunomodulators with allopurinol.

DISCUSSION

We sought to determine whether allopurinol, which competitively inhibits XO, could be used in MP/AZA non-responders to deliberately shunt metabolism of MP towards the active metabolites 6-TGN and 6-MMP, and in particular whether allopurinol could optimize 6-TGN production. To our knowledge this is the first report of this practice in IBD, although it has previously been reported in abstract form in the renal transplant literature[11] when low-dose allopurinol (25 mg on alternate days) was added to conventional 'triple therapy' immunosuppression (ciclosporin, prednisolone, AZA) in 12 adult renal transplant recipients. The number of acute rejection episodes was compared with 15 control patients receiving the same immunosuppressive regimen alone. There was only one rejection episode amongst the allopurinol-treated patients compared with 11 rejection episodes in the control group, and there was no difference in the incidence of infection in the two groups, although detailed statistics were not provided and the report predated the performance of thiopurine metabolite measurements.[11]

There are several possible mechanisms by which allopurinol may optimize thiopurine immunomodulator therapy, although none offer a completely satisfactory explanation. Logically, the inhibition of XO by allopurinol should lead to an increase in levels of both 6-TGN and 6-MMP. In contrast, however, we found that while 6-TGN levels increased, concentrations of 6-MMP declined; suggesting that TPMT was being inhibited. Allopurinol is not known to interact with TPMT, as illustrated by a recent *in vitro* experiment that demonstrated constant TPMT activity in RBC hemolysate despite addition of increasing concentrations of allopurinol (Prometheus Laboratories, unpublished data).

The aminosalicylates sulfasalazine and mesalazine (mesalamine) are known to non-competitively inhibit TPMT, and theoretically could increase 6-TGN levels when given with a thiopurine. However, Szumlanski and Weinshilboum[12] determined that the concentrations required to inhibit TPMT enzyme activity by 50% (IC_{50}) were 78 μM for sulfasalazine and 1240 μM for mesalazine. Given that a 2 g oral dose of sulfasalazine results in a serum concentration of the drug of 25–50 μM, substantially below the IC_{50} the potential inhibition is unlikely to be of clinical significance in a patient with normal or elevated TPMT activity. In our cohort, six of the 15 patients were also taking aminosalicylates, which may have produced some inhibition of TPMT, although again the same biochemical effect was seen in patients taking, or not taking, an aminosalicylate.

Another possible explanation for the effect of allopurinol lies with the reduced dose of thiopurine given once the allopurinol was commenced. Reports from the transplant literature consistently show that in patients treated with AZA, TPMT activity slowly increases during treatment, presumably due at least partly to

Table 1. Diagnosis and biochemical response of 15 patients before and after commencement of allopurinol therapy

	Diagnosis	Pre-/post-thiopurine dose (mg)	Pre-/post-6-TGN (pmol/8×10^8 RBC)	Pre-/post-6-MMP (pmol/8×10^8 RBC)	Pre-/post-WBC ($\times 10^8$/L)
Patient 1	UC	6-MP: 100/25	183/576	11 658/1546	9.1/2.7
Patient 2	IC	6-MP: 125/25	194/615	11 363/<LLD	7.8/5.8
Patient 3	IC	6-MP: 100/100	274/792	8816/1108	8.3/4.1
Patient 4	UC	6-MP: 75/50	178/427	8569/8714	13.0/8.6
Patient 5	CD	AZA: 50/50	192/277	15 961/<LLD	10.9/7.5
Patient 6	CD	6-MP: 100/50	134/230	2079/<LLD	5.9/5.2
Patient 7	UC	6-MP: 75/62.5	234/344	7352/1623	5.6/5.6
Patient 8	CD	6-MP: 75/25	211/416	17 573/<LLD	8.2/3.7
Patient 9	CD	6-MP: 75/50	116/438	9198/1334	6.6/5.4
Patient 10	CD	6-MP: 83.3/25	175/290	3199/<LLD	11.6/5.8
Patient 11	CD	6-MP: 100/100	126/288	11 279/6990	5.4/4.9
Patient 12	CD	6-MP: 100/50	143/326	16 344/2044	6.4/4.8
Patient 13	UC	AZA: 250/100	155/313	6799/1215	17.3/16.7
Patient 14	CD	AZA: 250/100	101/240	12 475/<LLD	5.1/6.7
Patient 15	CD	AZA: 200/100	370/385	17 542/4095	3.0/6.3

'Pre' and 'post' refer to timing in relation to commencement of allopurinol.
UC, ulcerative colitis; CD, Crohn's disease; IC, indeterminate colitis; MP, mercaptopurine; AZA, azathioprine; 6-TGN, 6-tioguanine; 6-MMP, 6-methylmercaptopurine; WBC, white blood cell count; LLD, lower limit of detection; TPMT, thiopurine methyltransferase.
TPMT activity was measured in patient 3 and patients 5–11 and was normal ($TPMT^H/TPMT^H$).
Patients 2, 4, 7, 9, 10 and 11 were taking concomitant aminosalicylate therapy.

enzyme induction, and then declines to pre-treatment levels when the AZA is withdrawn. One recent report showed that in 23 of 26 patients commenced on AZA, the TPMT activity increased by a mean of 92%.[13] Twelve of the 15 of our patients had the dose of MP/AZA reduced to 25–50% of the original dose, which could theoretically cause a reduction in TPMT activity and preferential shunting towards 6-TGN; although serial measurements of TPMT activity were not performed. It should be noted, however that in our cohort the biochemical response was seen equally in those patients that did, and those that did not, have doses of thiopurine reduced. Another possibility is that allopurinol alters metabolite distribution, rather than production. *In vitro* studies indicate that accumulation of metabolites in red blood cells occurs after hepatic metabolism of MP. Changes in red blood cell metabolite levels could therefore also occur due to allopurinol-induced changes in metabolite distribution, rather than production, although there is currently no data to confirm this.

The majority of patients appeared to clinically respond after the introduction of allopurinol to the immunomodulator therapy, although formal disease assessment was not performed. It may be possible that allopurinol had beneficial effects, independent from its effect on thiopurine metabolism. Allopurinol inhibits XO, serum levels of which are known to be elevated in inflammatory states. The products of oxidative hydroxylation by the enzyme include oxygen-free radicals which are implicated in tissue damage, so allopurinol could lead to reduced production of these potentially harmful reactive oxygen species.[14] Finally, there is *in vitro* data to suggest that allopurinol has inhibitory effects on TNF-α, one of the pivotal inflammatory mediators implicated in the pathogenesis of IBD. Allopurinol impaired the cytotoxic effects of human recombinant TNF-α against cultured cell lines, and also inhibited the production of TNF-α by stimulated human mononuclear cells.[15]

Whatever the mechanism, the addition of allopurinol to thiopurine non-responders with preferential shunting to 6-MMP metabolites appears to be an effective and safe means to shift metabolism towards 6-TGN. Careful monitoring of WBC is required to avoid leucopoenia. We performed weekly complete blood counts for 4 weeks after the addition of allopurinol and used serial metabolite measurements beginning at 2–4 weeks after allopurinol was added to guide subsequent dose adjustments. Future studies will determine the clinical safety and efficacy of adjunctive allopurinol to AZA or MP non-responders with unfavourable metabolite profiles. Clinical follow-up is currently underway to determine the efficacy of these metabolite adjustments and the safety, including the risk of veno-occlusive disease

and nodular regenerative hyperplasia, in patients induced to increasing 6-TGN levels.

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