Long term efficacy and safety of allopurinol and azathioprine or 6-mercaptopurine in patients with inflammatory bowel disease

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Abstract

Background and aims: We previously reported that IBD patients who are non-responders to thiopurines with preferential shunting of metabolites to hepatotoxic 6-methylmercaptopurine ribonucleotides compared to 6-thioguanine nucleotides can reverse the ratio of 6-MMP/6-TGN and respond to thiopurines with the addition of allopurinol. The objective of this study is to report long term efficacy and safety, along with results for an additional 11 patients.
Methods: Retrospective chart review of patients at the University of Chicago IBD Center treated with allopurinol in addition to thiopurines.
Results: Twenty five patients with Crohn's disease or ulcerative colitis were enrolled. Within the first month of therapy 6-TGN metabolite levels increased from a mean of 186.5 ± 17.4 (SE) to 352.8 ± 37.8 pmol/8×10^8 ($p = 0.0001$). Over the same period 6-MMP levels decreased from a mean of $11,966 \pm 1697$ to 2004 ± 536 pmol/8×10^8 ($p < 0.0001$). The mean daily dosage of prednisone decreased from 19.8 ± 3.8 mg to 5.3 ± 2.7 mg ($p = 0.03$). Thirteen patients have a minimum of one year follow-up. Nine of these thirteen patients have continued on therapy for at least 2 years. All thirteen of these patients continue to be in clinical remission at the last follow-up visit. No patients have had evidence of sustained thrombocytopenia or abnormal liver enzymes.
Conclusions: In AZA/6-MP non-responders with increased 6-MMP/6-TGN ratios, addition of allopurinol continues to demonstrate safety and efficacy for long-term maintenance and steroid-sparing in IBD.
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1. Introduction

Azathioprine and 6-mercaptopurine (6-MP) have demonstrable efficacy as a maintenance therapy after steroid[1−4] or biologic[5,6] induced remissions for Crohn's disease and after steroid-induced remission in ulcerative colitis.[7,8] Although, numerous enzymes are involved in the metabolism and catabolism of thiopurines, thiopurine S-methyltransferase (TPMT) is considered a key enzyme that diverts 6-mercapto-purine catabolism towards 6-methylmercaptopurine nucleo-tides (6-MMP) and away from the presumptive active 6-thioguanine nucleotides (6-TGN).[9] 6-TGN inhibits purine nucleotide metabolism by interrupting lymphocyte prolifera-tion[10] and more recently phosphorylated 6-TGNs have been found to induce apoptosis of T lymphocytes via Rac-1 inhibition[11] and to downregulate proinflammatory genes in activated T lymphocytes.[9,12]

TPMT production and enzyme activity is determined by genetic polymorphisms. A significant proportion of patients who do not respond to thiopurines are characterized by suboptimal 6-TGN and preferential 6-MMP production.[13,14] Previous work at our institution has shown that the addition of allopurinol, a xanthine oxidase inhibitor, to thiopurine non-responders with high 6-MMP metabolite levels asso-ciated with transaminitis increased 6-TGN production, reduced 6-MMP levels, normalized liver enzymes, and led to a reduction in disease activity in patients with both Crohn's disease and ulcerative colitis.[15,16] There have been three further studies in adult[17,18] and pediatric IBD patients with similar results.[19]

As 6-TGN is myelotoxic it is important to determine the risks of short and long term myelosuppression. Further-more, thiopurine therapy (azathioprine, 6-thioguanine) has been associated with an increased risk of liver damage in the form of nodular regenerative hyperplasia (NRH) and veno-occlusive disease (VOD).[20,21] Non-cirrhotic portal hypertension associated with NRH may manifest as throm-bocytopenia secondary to splenomegaly prior to clinical symptoms and signs. The association of thiopurine therapy with NRH and VOD may be related to increased levels of the active metabolites of thiopurine therapy. As co-therapy with allopurinol has been shown to increase efficacy likely via increasing active metabolites, it is important to assess the long term potential for hepatotoxicity from the combination of allopurinol and thiopurines. Therefore, we describe the clinical efficacy and safety of an expanded cohort of patients treated with allopurinol in addition to reduced dose thiopurine therapy.

2. Materials and methods

Twenty five predominantly steroid-dependent outpatients from one tertiary referral IBD clinic who were non-responders to 6-MP or azathioprine with 6-TGN levels less than 235 pmol/8×10^8 erythrocytes and 6-MMP levels greater than 5700 pmol/8×10^8 erythrocytes are described. Patients were considered steroid dependent if there was clinical relapse during weaning of corticosteroids or if they required more than one course of corticosteroids to re-induce remission. Fifteen patients had CD, 8 had UC and 2 had indeterminate colitis (IC). Patients were experiencing active disease or requiring corticosteroids at the time of inclusion into the study. Seven patients were also receiving aminosalicylates. Three of 25 patients underwent TPMT genotype measurement prior to study inclusion; all were wildtype TPMT. One patient had TPMT enzyme activity measured (phenotype) and this was in the normal range. Metabolite measurements were performed by Prometheus Laboratories with high-performance liquid chromatography methods.[22,23]

All patients received 100 mg of allopurinol daily, with the dose of thiopurine immediately reduced to 25−50% of the original dose. Steroids were tapered depending on clinical response as determined by the treating physician. Complete blood counts (CBC) and liver enzymes were closely mon-itored. CBC was measured weekly for the first month, biweekly for the second month and then every 3 months. Liver enzymes were measured within the first month of treatment and then every 3 months. 6-TGN and 6-MMP were measured by Prometheus Labs (San Diego, CA) two to four weeks after initiating allopurinol. Clinical efficacy was assessed by the Harvey Bradshaw Index for CD patients[24] and by the partial Mayo score[25] (without sigmoidoscopy) for UC and IC patients. Short term clinical efficacy was assessed at 3 months. Long term clinical efficacy was assessed at their last clinic visit or, if they stopped combination therapy, at the last visit prior to stopping the combination therapy. Clinical efficacy scores and adverse events were obtained from detailed review of outpatient medical records. All complete blood counts and liver enzyme measurements obtained during combination therapy were prospectively recorded. Platelet count and liver enzymes were used as surrogate markers to assess for presence of liver disease. Liver ultrasound and liver biopsies were not performed.

The results are presented as mean±SE unless otherwise stated. The nonparametric Wilcoxon signed rank test was used to assess the statistical significance of changes in the level of metabolites, transaminases, corticosteroid dose, and disease activity before and after the administration of allopurinol. The signed rank test was used because paired data with unequal variance is present. When 6-MMP levels were not detected by the assay, the lower limits of detection was assumed. Leukopenia was defined as WBC $<3.5 \times 10^9$/L and thrombocytopenia was defined as a platelet count $<150 \times 10^9$/L. Subjects were excluded from individual ana-lyses if either the pre- or post-allopurinol data were missing. 6-mercaptopurine dose was converted to azathioprine dose by a factor of 2. Statistical analysis was performed with Stata SE 10.0 (Stata Corporation, College Station, TX). Approval from the University of Chicago institutional review board was obtained prior to any data collection.

3. Results

Fourteen patients were initially enrolled from the University of Chicago.[16] In this group, 12/14 subjects (5M/7F) had follow-up data. Nine of the twelve patients are continuing on allopurinol after a mean of 36.1 months (range 22−50 months). The reasons for discontinuation were: surgery (2), and pancytopenia (1). Since the initial protocol, 11 additional patients have started allopurinol in combination with thiopurine therapy. All 11 patients (5M/6F) continue to

be on combination therapy after a mean of 12.1 months (range 4–34 months). The mean dose of 6-mercaptopurine and azathioprine before starting allopurinol was 114 ± 10.3 mg and 193 ± 17.4 mg respectively. After starting allopurinol the mean dose for 6-mercaptopurine was 51 ± 8.8 mg and for azathioprine 69 ± 6.4 mg. The mean weight based dose of pre-allopurinol thiopurine therapy was the equivalent of 2.87 mg/kg/day of azathioprine. Baseline clinical characteristics of the patients are described in Table 1.

3.1. Metabolite levels and liver enzymes

Within the first month of therapy 6-TGN metabolite levels increased from a mean of 186.5 ± 17.4 to 352.8 ± 37.8 pmol/8×10^8 RBC ($p=0.0001$). Over the same period 6-MMP levels decreased from a mean of $11,966 \pm 1697$ to 2004 ± 536 pmol/8×10^8 RBC ($p<0.0001$) (Fig. 1A and B). Mean AST decreased from 39.6 ± 7.3 to 24.2 ± 1.6 ($p=0.08$), and mean ALT decreased from 82.0 ± 31.9 to 27.5 ± 4.8 ($p=0.04$) (Fig. 2).

3.2. Steroid sparing

Seventeen patients were on steroids prior to initiation of allopurinol (10 CD, 7 UC/IC). Twelve were on prednisone and five were on budesonide. Duration of steroid treatment prior to allopurinol therapy was calculated for 12 patients with a mean duration of 7.2 ± 8.3 months. Duration of steroid treatment could not be calculated for the remaining patients as they had been on episodic steroid therapy for many years. Ten patients (10/17) stopped steroids completely with seven patients stopping within 3 months (7/10). The three remaining patients (3/10) were completely off steroids by 6 months. Eight of the twelve patients who were on prednisone are completely off any form of steroids. The mean daily dosage
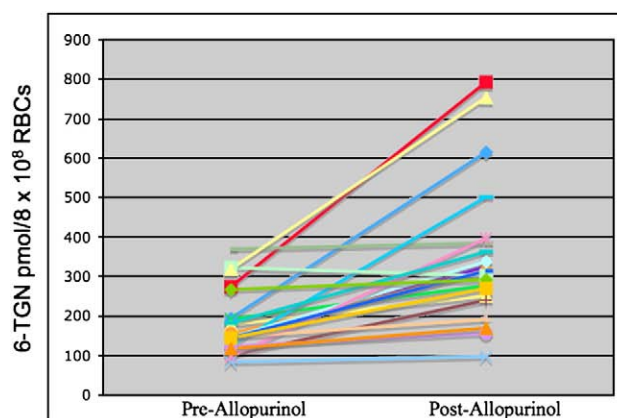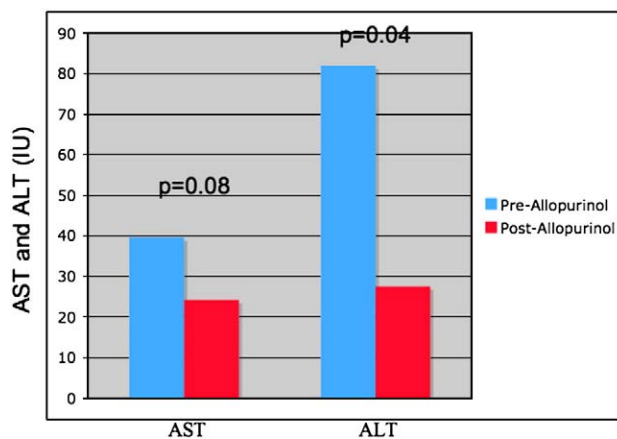

A) Increase in 6-TGN after allopurinol
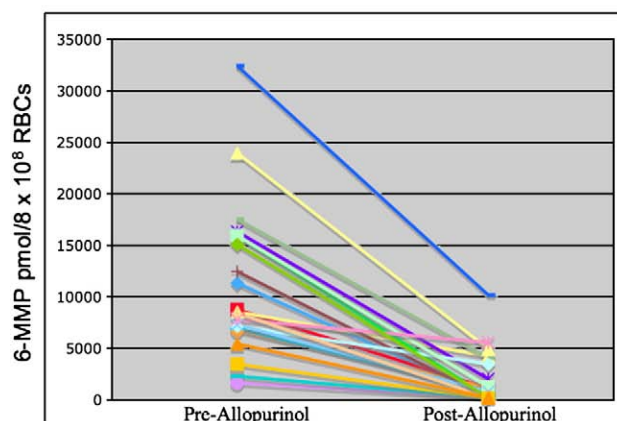
B) Decrease in 6-MMP after allopurinol

Figure 1   Metabolite levels before and after allopurinol. A: 6-TGN, 6-thioguanine nucleotides. B: 6-MMP, 6-methylmercaptopurine nucleotides.

of prednisone decreased from 19.8 ± 3.8 mg to 5.3 ± 2.7 mg ($p=0.03$). The mean daily dosage of budesonide decreased from 7.5 ± 0.7 mg to 4.5 ± 1.9 mg ($p=0.40$).
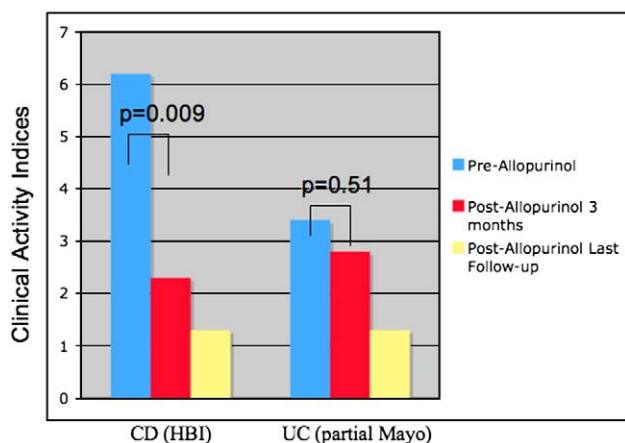
Table 1 Baseline clinical characteristics.	
Mean age $\pm$ SD (yr)	41 ± 14.3
Gender, n (% of patients)	
Male	12 (48%)
Diagnosis, n	
CD	15
UC	8
IC	2
Mean disease duration + SD (yr)	15 ± 12.9
History of surgery, n (% of patients)	6 (24%)
Steroid therapy	
Prednisone	12 (48%)
Budesonide	5 (20%)
Thiopurine therapy	
Azathioprine	14 (56%)
6-mercaptopurine	11 (44%)
Concomitant medications, n (%of patients)	
5-ASA	7 (28%)
Anti-TNF	1 (4%)

SD, standard deviation; yr, years; n, number of patients; CD, Crohn's disease; UC, ulcerative colitis; IC, indeterminate colitis; 5-ASA, aminosalicylate; Anti-TNF, anti-tumor necrosis factor.

Figure 2   Liver enzymes before and after allopurinol. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Figure 3 Clinical efficacy short and long term. CD, Crohn's disease; UC, ulcerative colitis; HBI, Harvey Bradshaw Index.

3.3. Short term clinical efficacy

Patients with UC/IC had a decreased partial Mayo score from a mean of 3.4 ± 0.8 to 2.8 ± 0.7 points ($p = 0.51$) over the first 3 months after the addition of allopurinol. Patients with CD had decreased HBI scores from 6.2 ± 1.5 to 2.3 ± 0.7 ($p = 0.009$) over the first 6 months (Fig. 3).

3.4. Long term clinical efficacy

Thirteen patients have a minimum of one year follow-up. Nine of these thirteen patients have continued on therapy for at least 2 years. Four of these nine have been on both allopurinol and thiopurine therapy for over 40 months. Of the thirteen patients who have been on allopurinol and thiopurine for at least 1 year, nine were initially on steroids and six of these patients continue to be steroid free during their entire follow-up period with only one patient requiring escalation to biologic therapy. All thirteen of these patients continue to be in clinical remission. The mean partial Mayo score was 1.3 ± 0.8, and the mean HBI score was 1.3 ± 0.6 at the last follow-up visit.

3.5. Adverse events

There have been six subjects who developed leucopenia, all with prompt recovery after reduction of the thiopurine dose, without any episodes of fever or opportunistic infection. There are no patients with sustained thrombocytopenia, and only one patient developed transient thrombocytopenia when the dose of 6-MP was increased without medical advice. There have been no cases of new onset of abnormal liver enzymes. There have been no side effects secondary to allopurinol itself (hypersensitivity reaction, rash or gastrointestinal side effects).

4. Discussion

In AZA/6-MP non-responders with increased 6-MMP/6-TGN ratios the addition of allopurinol continues to demonstrate both short-term and long-term safety and efficacy. Further-

more, this regimen with careful monitoring has been shown to be safe over a mean follow-up time of 3 years, and up to 4 years in a smaller subset of our patients. There have been no cases of sustained thrombocytopenia or abnormal liver enzymes. Any leucopenia encountered was mild and reversible.

The addition of allopurinol increased 6-TGN and decreased 6-MMP and hepatic transaminases. The mean 6-TGN increased to 353 pmol/8×10^8 erythrocytes. Previous studies have found a positive correlation between 6-TGN levels and clinical efficacy[26–28] and a meta-analysis of twelve studies found that patients were more likely to be in remission (odds ratio 3.3) above a threshold level of 230–260 pmol/8×10^8 erythrocytes.[29] Others have reported similar metabolic and clinical responses for patients treated by combination therapy with thiopurines and allopurinol.[17–19]

Change in clinical efficacy using the partial Mayo score was not significant in UC/IC patients. However, the baseline score was calculated with patients already on steroid therapy. Therefore the steroid-sparing effect of allopurinol and thiopurines is likely a better marker of clinical efficacy. There was a significant decrease in the mean dose of prednisone from 20 mg daily to 5 mg daily, a dose associated with less systemic toxicity. In our entire cohort two-thirds of the patients on prednisone were able to discontinue steroids and remain steroid-free at their last follow-up visit.

The addition of allopurinol to thiopurine therapy has been studied in cancer[30], renal transplant[31], autoimmune pancreatitis[32] and more recently in IBD pediatric patients.[19] 6-MP is metabolized by 3 competing pathways: methylation by TPMT to 6-MMP, oxidation by xanthine oxidase to 6-thiouric acid and conversion by HPRT (hypoxanthine phosphoribosyl-transferase) to 6-thioinosine monophosphate (6-TIMP), with subsequent production of 6-TGN. The mechanism of the metabolic interaction with allopurinol remains unknown. The possibility that active IBD impacts on mucosal xanthine oxidase activity has been refuted by Ansari et al.[33] although Wong identified a single patient who was unable to produce adequate levels of 6-TGN due to increased xanthine oxidase activity that was successfully inhibited by allopurinol.[32] Although allopurinol's primary mechanism is inhibition of xanthine oxidase, if this were the sole mechanism both 6-MMP and 6-TGN would be expected to rise. Duley et al. hypothesized that allopurinol, via its metabolites such as oxypurinol, inhibits TPMT.[34] Oxypurinol is converted to oxypurinol riboside monophosphate, a 6-oxo analogue of 6-TIMP, which is also methylated by TPMT. However in vitro and in vivo TPMT inhibition by allopurinol, or oxypurinol, has not been confirmed.[15,16] Alternatively, allopurinol, as a purine analog, may be converted to metabolites that have an effect on proliferation of lymphocytes. Ansari et al. suggest that the increase in 6-TGN is secondary to inhibition of first-pass metabolism by xanthine oxidase therefore increasing the bioavailability of the thiopurine. The decreased thiopurine dose is suboptimal for the induction of TPMT activity that otherwise may occur, hence the reduction of 6-MMP.[18] A final hypothesis is that there are additional effects of allopurinol apart from the metabolic interaction. Allopurinol has been studied for its role as an oxygen-derived free radical scavenger in UC,[35,36] and pouchitis,[37] with mixed results.

There are several limitations to this study that need to be acknowledged. Our study did not have liver biopsies to look for hepatic damage. However from a clinical perspective

there was no evidence of hepatic toxicity. Metabolite levels at the last follow-up visit were not available in order to see if there were differences between patients who discontinued the allopurinol versus those that continued. However there were only three patients that discontinued allopurinol co-therapy. TPMT genotyping or phenotyping was not done in the majority of the patients. They were not done as allopurinol is not an inhibitor of TPMT activity and the patients' previous tolerance of thiopurines ruled out patients with absent/low TPMT activity.

In summary, allopurinol, with careful monitoring, can be safely added to thiopurine therapy in non-responders with elevated 6-MMP and low 6TGN levels for long term maintenance therapy of IBD. Given that patients who fail azathioprine maintenance would likely need further escalation in therapy with the potential for other adverse events we believe that this strategy continues to be an appropriate therapeutic maneuver in selected patients who are compliant with close follow-up. Prospective studies are planned to further validate the safety and efficacy of this therapeutic maneuver.

Acknowledgements

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