

Effect of Allopurinol on Clinical Outcomes in Inflammatory Bowel Disease Nonresponders to Azathioprine or 6-Mercaptopurine

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See editorial on page 170; and see Su C et al on page 516 and D'Haens G et al on page 763 in the February 2007 issue of *Gastroenterology*.

Background & Aims: Many IBD patients not responding to azathioprine (AZA) or 6-mercaptopurine (6-MP) preferentially metabolize 6-MP to 6-methylmercaptopurine (6-MMP). We describe the use of allopurinol in AZA/6-MP nonresponders to deliberately shunt metabolism of 6-MP toward 6-thioguanine (6-TGN) and improve clinical responses. **Methods:** Twenty outpatients who were AZA/6-MP nonresponders and had high 6-MMP metabolite levels were included. Subjects were commenced on allopurinol 100 mg daily, and the dose of 6-MP/AZA was reduced to 25%–50% of the original dose. **Results:** After allopurinol was started, mean 6-TGN levels increased from 191.3 (\pm standard error of the mean) \pm 17.1 to 400.3 \pm 36.9 pmol/8 \times 10⁸ red blood cells ($P < .001$), whereas mean 6-MMP levels decreased from 10,604.7 \pm 1278.2 to 2000.6 \pm 437.1 pmol/8 \times 10⁸ red blood cells ($P < .001$). The addition of allopurinol led to a reduction in the mean partial Harvey Bradshaw Index in Crohn's disease patients from 4.9 \pm 1.0 to 1.5 \pm 0.3 points ($P = .001$), and in ulcerative colitis patients mean Mayo Scores decreased from 4.1 \pm 0.7 to 2.9 \pm 0.7 points ($P = .13$). The addition of allopurinol enabled a reduction in mean daily prednisone dosage from 17.6 \pm 3.9 to 1.8 \pm 0.7 mg ($P < .001$) and led to normalization of transaminase levels, with mean AST levels reducing from 42.5 \pm 8.1 to 23.5 \pm 1.6 IU ($P = .12$) and mean ALT levels reducing from 101.6 \pm 26.9 to 33.9 \pm 5.2 IU ($P = .01$). **Conclusions:** The addition of allopurinol to thiopurine nonresponders with high 6-MMP metabolite levels is an effective and safe means of optimizing 6-TGN production, leading to improved disease activity scores, reduced corticosteroid requirements, and normalization of liver enzymes.

The thiopurine immunomodulators azathioprine (AZA) and 6-mercaptopurine (6-MP) are now an integral part of the therapeutic armamentarium used for the treatment of inflammatory bowel disease (IBD). In Crohn's disease (CD) there is evidence to support the use of AZA/6-MP for maintaining remissions, as steroid-sparing agents, and for the treatment of perianal fistulas.^{1–3} In UC, controlled trial evidence supports the use of thiopurines as steroid-sparing agents and for the maintenance of remission, with possible benefits seen in the

induction of remission in chronic active steroid-refractory disease.⁴

Recent advances in pharmacogenomics and therapeutic monitoring of these agents have allowed for more accurate and aggressive dosing of immunomodulators to improve the likelihood of response. Nevertheless, up to 50% of patients do not respond to an adequate dose and duration of therapy with these drugs, with response defined as the induction and maintenance of a corticosteroid-free remission, and the probability of response appears to be due to genetically determined individual variations in drug metabolism. AZA and 6-MP are both inactive prodrugs that are metabolized via 3 main enzymatic pathways to produce the nucleotide metabolites 6-thioguanine (6-TGN), 6-methylmercaptopurine (6-MMP), and 6-thiouracil (6-TU) (Figure 1). 6-TGN appears to be the active metabolite responsible for therapeutic efficacy and the risk of myelotoxicity, whereas 6-MMP levels are independent of efficacy but correlate with the risk of hepatotoxicity in the form of elevation of hepatic transaminase levels.⁵

Important recent work has increased the understanding of the role of the 6-TGN metabolites in achieving therapeutic efficacy. Three thioguanine nucleotides are recognized (6-thioguanine monophosphate [6-TGMP], 6-thioguanine diphosphate [6-TGDP], and 6-thioguanine triphosphate [6-TGTP]) and are distinguished by the number of phosphate residues attached during the anabolic conversion of 6-thiosine 5'-monophosphate. It has previously been assumed that the immunosuppressive actions of thiopurines are achieved via the incorporation of 6-TGN into lymphocytic DNA, thereby inhibiting cellular proliferation.⁶ However, Tiede et al⁷ have suggested an alternative mechanism of action of these drugs by showing that the 6-TGTP metabolite of AZA/6-MP actively stimulates apoptosis of lamina propria T lymphocytes by binding to and

Abbreviations used in this paper: AZA, azathioprine; CD, Crohn's disease; GMPS, guanosine monophosphate synthetase; HPRT, hypoxanthine phosphoribosyltransferase; IBD, inflammatory bowel disease; IC, indeterminate colitis; IMPDH, inosine monophosphate dehydrogenase; 6-MMP, 6-methylmercaptopurine; 6-MP, 6-mercaptopurine; pHBI, partial Harvey Bradshaw Index; RBC, red blood cell; 6-TGDP, 6-thioguanine diphosphate; 6-TGMP, 6-thioguanine monophosphate; 6-TGN, 6-thioguanine nucleotide; 6-TGTP, 6-thioguanine triphosphate; 6-TXMP, 6-thioxanthosine monophosphate; TNF- α , tumor necrosis factor- α ; TPMT, thiopurine methyltransferase; 6-TU, 6-thiouracil; UC, ulcerative colitis; WBC, white blood cell; XO, xanthine oxidase.

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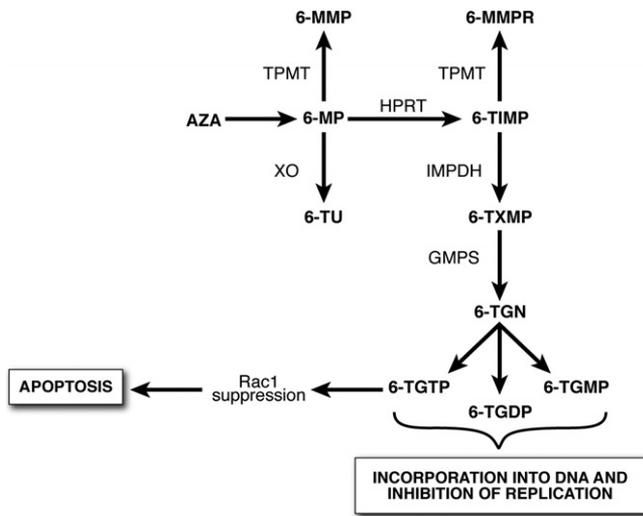


Figure 1. Metabolism of 6-mercaptopurine. (HPRT, hypoxanthine phosphoribosyltransferase; 6-TIMP, 6-thiosine 5'-monophosphate; IMPDH, inosine monophosphate dehydrogenase; 6-TXMP, 6-thioxanthosine monophosphate; GMPS, guanosine monophosphate synthetase).

suppressing Rac1, an intracellular enzyme involved in the activation of the nuclear factor- κ B and STAT-3 pathways.

In equating metabolite concentrations to clinical response, in the initial retrospective series, the frequency of therapeutic response increased at 6-TGN levels greater than $235 \text{ pmol}/8 \times 10^8$ erythrocytes, and transaminase elevation correlated with 6-MMP levels greater than $5700 \text{ pmol}/8 \times 10^8$ erythrocytes.⁵ A more recent study provided further evidence that it might be the 6-TGTP moiety that is responsible for therapeutic efficacy. In 47 CD patients receiving AZA, the thioguanine nucleotide metabolites were found to be composed of 80% 6-TGTP, 16% 6-TGDP, and less than 1% 6-TGMP. However, in almost half the patients there was a disproportionately high production of 6-TGDP to greater than 15% of the total 6-TGN concentration and therefore less 6-TGTP production. In this subgroup with lower 6-TGTP levels, clinical response rate was significantly lower, and the number of flares per year and infliximab use were significantly higher.⁸ A previous study further highlighted that there appears to be different dominant metabolic pathways among patients who do or do not respond to dose escalation of thiopurines. In this study only 27% of patients responded to thiopurine dose escalation by increasing 6-TGN levels into the therapeutic range, whereas nonresponders instead preferentially metabolized the drugs toward 6-MMP production.⁹

Taken together, these studies show that a desirable metabolic profile of thiopurine drug metabolism in an individual would be one that maximizes 6-TGN production while minimizing 6-MMP production. It has been shown that 6-TGN concentrations are inversely correlated with functional activity of the thiopurine methyltransferase (TPMT) enzyme, whereas elevations in 6-MMP are associated with high, functional TPMT activity.⁵ Functional activity of TPMT is genetically determined, and population studies show a tri-modal distribution of activity; 89% of the population possess normal or high activity (homozygous high), 11% have intermediate activity (heterozygotes), and 0.3% have low or absent activity of the enzyme

(homozygous low).¹⁰ Much attention has deservedly been given to the homozygous low group of patients with minimal TPMT activity in whom prompt leukopenia will occur with low doses of thiopurine immunomodulators, and in whom these drugs should be avoided or used extremely cautiously in very low doses. Indeed, patients with intermediate TPMT activity have been found to be the most responsive to low-dose thiopurine therapy. However, of potentially equal (or greater) importance is the group of patients with high TPMT activity who, in contrast, preferentially metabolize the drugs toward 6-MMP, thereby reducing 6-TGN production and therapeutic efficacy. It would appear that at least 10% of patients fall into this category of high functional TPMT activity and on dose escalation develop a disproportionately high 6-MMP:6-TGN ratio.^{11,12}

Our group has recently demonstrated that in IBD patients displaying a high TPMT activity metabolite profile (6-MMP > 6-TGN), the addition of low doses of the xanthine oxidase (XO) inhibitor allopurinol can safely and effectively optimize 6-TGN production and reduce that of 6-MMP. In our initial report, the addition of allopurinol 100 mg daily in combination with an immunomodulator dose reduction of 50%–75% led to an increase in 6-TGN from a mean of 185.73 ± 17.7 to $385.4 \pm 41.5 \text{ pmol}/8 \times 10^8$ red blood cells [RBCs] ($P < .001$) and a reduction in 6-MMP from a mean of $10,380 \pm 1245$ to $1732 \pm 502 \text{ pmol}/8 \times 10^8$ RBCs ($P < .001$). With close monitoring of white blood cell (WBC) counts this maneuver could be performed safely, and any mild leukopenia encountered was reversible on further thiopurine dose reduction.¹³ We now report the clinical outcomes of this therapeutic maneuver by demonstrating improvements in disease activity scores, corticosteroid requirements, and abnormal liver enzymes with the metabolite switch induced by the addition of allopurinol.

Materials and Methods

Patient Population

Twenty predominantly steroid-dependent outpatients from 2 tertiary referral IBD clinics who were nonresponders to 6-MP or AZA with 6-TGN levels less than $230 \text{ pmol}/8 \times 10^8$ erythrocytes and 6-MMP levels greater than $5000 \text{ pmol}/8 \times 10^8$ erythrocytes are described. Twelve patients had CD, 6 had UC, and 2 had indeterminate colitis (IC). All patients had active disease or were requiring corticosteroids to remain in clinical remission at the time of the addition of allopurinol. Sixteen patients were receiving corticosteroids at the time of inclusion into the study. Five patients were also receiving aminosalicylates. TPMT activity had been previously measured before commencement of thiopurine therapy in 7 of the 20 patients and was normal (TPMT^H/TPMT^H) in all cases.

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. A low dose of adjunctive allopurinol was chosen to minimize the potential for toxicity from this combination therapy, particularly in the form of leukopenia, and the thiopurine dose reduction was determined from recommendations from the transplant and rheumatologic literature.^{14,15} In 4 patients the thiopurine dose was not reduced after the addition of allopurinol. Complete blood counts were obtained on a weekly basis for the first month of therapy and then every other week for the next month, and the 6-MP metabolites 6-TGN and 6-MMP were re-measured by

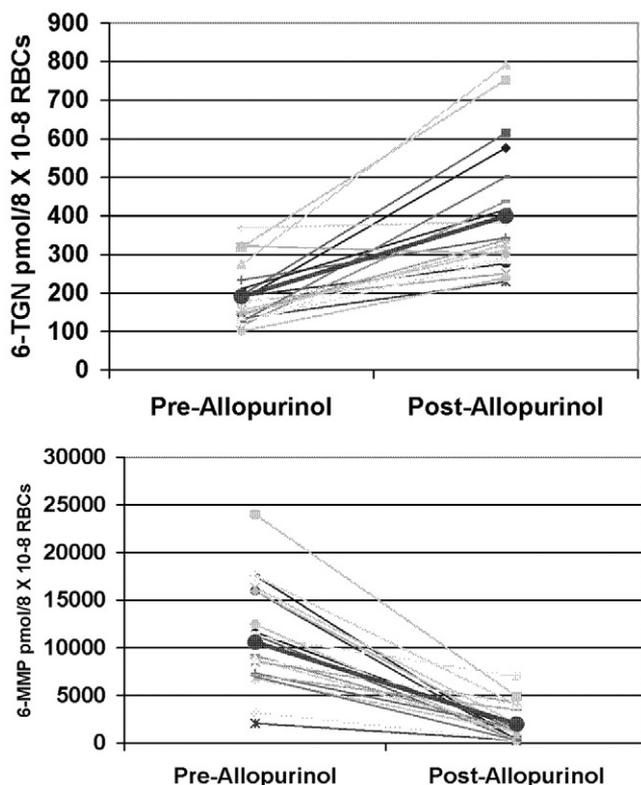


Figure 2. (A) Increase in 6-TGN (pmol/8 × 10⁸ RBCs) after the addition of allopurinol. (B) Decrease in 6-MMP (pmol/8 × 10⁸ RBCs) after the addition of allopurinol.

Prometheus Laboratories (San Diego, CA) approximately 2–4 weeks after initiating allopurinol. Disease activity assessments recorded 3 months after the addition of allopurinol were retrospectively obtained from detailed outpatient records that provided accurate data to calculate partial disease activity scores for both CD and UC. Partial Harvey Bradshaw Index (pHBI) scores (general well-being, abdominal pain, and number of liquid stools per day) were used to assess clinical response in CD patients,¹⁶ and partial Mayo Scores (without proctosigmoidoscopy) were used to assess patients with UC and IC.¹⁷

Outcome Measures and Statistical Analysis

To analyze the effects of allopurinol on thiopurine metabolism, corresponding values of 6-TGN and 6-MMP levels (pmol per 8 × 10⁸ RBCs) and WBC counts (× 10⁸/L), before and after commencing allopurinol, were recorded and compared by using the nonparametric Wilcoxon signed rank test. Values recorded indicate mean values and the standard error of the mean. When 6-TGN and 6-MMP levels were not detected by the assay, the lower limits of detection of 9 pmol/8 × 10⁸ RBCs and 294 pmol/8 × 10⁸ RBCs, respectively, were used in the statistical calculations. Similarly, to quantify the clinical benefits of the therapeutic maneuver, disease activity indices, corticosteroid dosages, and hepatic transaminase levels, before and after the addition of allopurinol, were recorded and compared by using the Wilcoxon signed rank test. Statistical analysis was performed with Stata SE 8.0 (Stata Corporation, College Station, TX).

Results

Before starting allopurinol, 5 patients were receiving AZA at a median dose of 200 mg daily, whereas 15 patients were receiving 6-MP at median daily doses of 87 mg. After commencement of allopurinol, the dose of AZA was reduced to a median dose of 90 mg daily, and 6-MP was reduced to a median daily dose of 51 mg.

After the addition of allopurinol, 6-TGN levels increased from a mean of 191.3 (± standard error of the mean) ± 17.1 to 400.3 ± 36.9 pmol/8 × 10⁸ RBCs (*P* < .001) (Figure 2A), whereas 6-MMP levels decreased from a mean of 10,604.7 ± 1278.2 to 2000.6 ± 437.1 pmol/8 × 10⁸ RBCs (*P* < .001) (Figure 2B). After allopurinol was added, 19 of 20 patients increased their 6-TGN level, with all but 1 patient achieving a 6-TGN level of greater than 235 pmol/8 × 10⁸ RBCs, and 5 patients achieved a 6-TGN level of greater than 500 pmol/8 × 10⁸ RBCs. Similarly, after allopurinol was added, all 20 patients reduced their 6-MMP level. The addition of allopurinol led to a decrease in WBCs from a mean of 8.8 ± 1.0 to 6.0 ± 0.7 × 10⁸/L (*P* < .001). Five patients developed leukopenia (defined as WBCs < 3.5 × 10⁸/L) that resolved with thiopurine dose reduction. Nadir WBC counts in these 5 patients ranged from 1.1–2.9 × 10⁸/L; however, no episodes of sepsis occurred. The biochemical response to allopurinol was seen equally in all groups regardless of diagnosis, whether CD, UC, or IC, and was also seen equally in patients taking and not taking concurrent aminosalicylates.

Assessment of disease activity in the 12 CD patients revealed a reduction in pHBI from a mean of 4.9 ± 1.0 points to 1.5 ± 0.3 points (*P* = .001) after the addition of allopurinol, and all but 1 patient exhibited an improvement in disease activity score. With a pHBI of less than 3 points to define remission, 7 of 12 patients with previously active disease entered remission with the addition of allopurinol. In UC patients, partial Mayo Scores fell from a mean of 4.1 ± 0.7 to 2.9 ± 0.7 points (*P* =

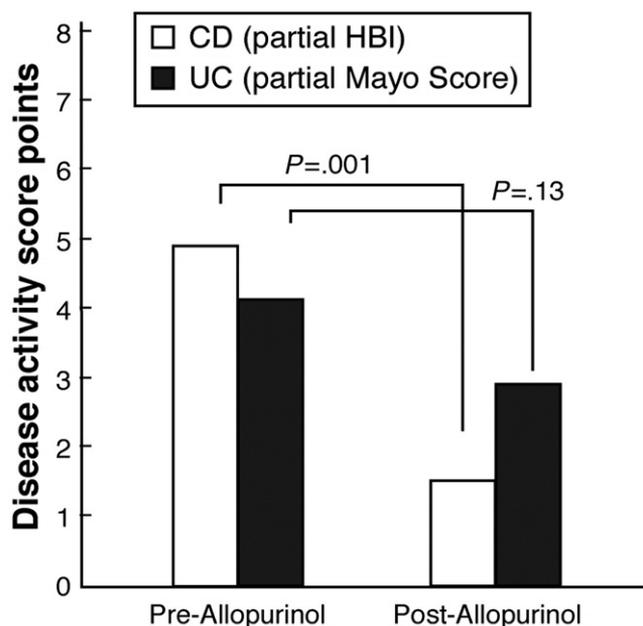


Figure 3. Reduction in disease activity scores after the addition of allopurinol.

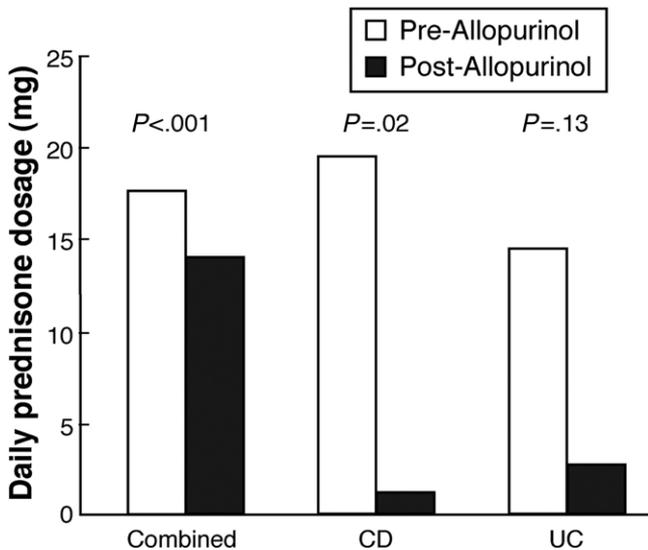


Figure 4. Reduction in mean daily prednisone dosage (mg) after the addition of allopurinol in the combined cohort, CD and UC patients.

.13) after the addition of allopurinol, and 4 of 6 patients exhibited improved disease activity scores (Figure 3). In the 2 IC patients, disease activity improved in one patient and was unchanged in the other with the addition of allopurinol.

Sixteen patients were receiving corticosteroids at the time of inclusion into the study, including 9 CD patients, all 6 UC patients, and 1 IC patient. Overall, the addition of allopurinol enabled a reduction in prednisone dosage from a mean daily dose of 17.6 ± 3.9 mg to 1.8 ± 0.7 mg ($P < .001$) after a period of approximately 3 months. Among the 9 CD patients, mean daily prednisone dosages reduced from 19.5 ± 5.9 mg to 1.3 ± 0.9 mg ($P = .02$), whereas among the 6 UC patients, mean daily prednisone dosages reduced from 14.5 ± 3.6 mg to 2.8 ± 1.4 mg ($P = .13$) (Figure 4). In the single IC patient prednisone dosage was reduced from 12.5 to 2.5 mg daily. Eight patients, 5 CD and 3 UC, were able to discontinue corticosteroids completely only after the addition of allopurinol.

Thirteen patients initially had biochemical evidence of hepatic transaminitis that is associated with preferential metabolism of thiopurines to 6-MMP metabolites.⁵ Eight patients had an elevated AST level, and 13 patients had an elevated ALT level, with elevations of ALT being more significant than of AST. The addition of allopurinol led to normalization of transaminase levels, with mean AST levels reducing from 42.5 ± 8.1 to 23.5 ± 1.6 IU ($P = .12$) and mean ALT levels reducing from 101.6 ± 26.9 to 33.9 ± 5.2 IU ($P = .01$) (Figure 5). These changes were seen equally among all diagnoses and among patients taking both AZA and 6-MP. After the addition of allopurinol, a reduction in AST level occurred in 7 of 8 patients, and a reduction in ALT level occurred in 5 of 13 patients. The addition of allopurinol in the 13 patients with baseline elevation of either AST or ALT led to normalization of one or both enzymes in 11 cases.

Despite an appropriate biochemical response to the addition of allopurinol, 3 patients required escalation to tumor necrosis factor- α (TNF- α) inhibitors, and an additional 2 patients required surgery to achieve remission during a follow-up period of 18 months, which represents 25% of the studied cohort. No patients developed adverse events directly attributable to the allopurinol itself, such as skin rash or renal impairment.

Discussion

Our group has previously demonstrated the favorable metabolic switch and 6-TGN optimization induced by the combination of low-dose allopurinol and thiopurine immunomodulators. This report of this combination describes clinical benefits in IBD patients, although a prior publication from the renal transplant literature described the use of 25 mg of allopurinol on alternate days added to conventional immunosuppressive therapy (cyclosporine, 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 1 rejection episode among the allopurinol-treated patients compared with 11 rejection episodes in the control group, and there was no difference in the incidence of infection between the 2 groups.¹⁸ Although nucleotide metabolite measurements were not performed, this report provided initial evidence of the potential to improve the efficiency of immunomodulator therapy by favorably altering the metabolism of these drugs.

Our results also showed that the addition of allopurinol to AZA/6-MP can safely improve the clinical efficacy parameters of immunomodulators in IBD patients, including improvements in disease activity scores and corticosteroid-sparing properties, presumably by the optimization of 6-TGN nucleotide metabolites. Evidence to support the efficacy of AZA/6-MP in IBD is stronger in CD than in UC,⁴ and our results are consistent with this understanding in that improvements in disease activity scores and corticosteroid-sparing properties reached statistical significance in CD patients, whereas in UC patients a significant reduction in corticosteroid dosage was achieved, while only a trend toward improvement in disease activity scores was seen. The corticosteroid-sparing capabilities of this combination showing that half the patients initially on steroids were able to completely taper them after the addition of allopurinol is a significant result because the lack of efficacy and adverse effects of long-term steroids in IBD are well-recognized, including increasing the risk of extramural complications of CD.¹⁹ The

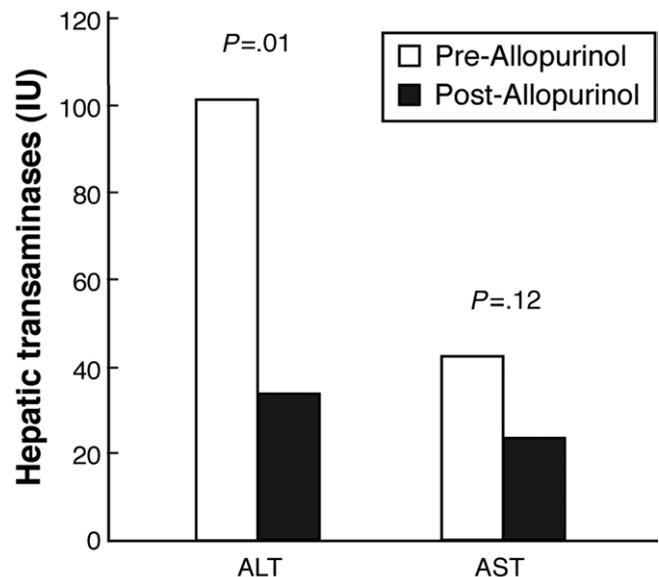


Figure 5. Reduction in mean hepatic transaminase levels (ALT, AST [IU]) after the addition of allopurinol.

reduction in transaminase levels seen with this combination is presumably due to the decrease in 6-MMP metabolites that occurs after the addition of allopurinol. Previously a significant subgroup of patients have been considered refractory to AZA/6-MP as a result of the elevation of hepatic transaminase levels, with or without symptoms such as nausea, that developed on dose escalation of these drugs, and this combination might allow these patients to benefit from immunomodulator therapy without the risk of hepatotoxicity.⁹

There are several possible means by which allopurinol might optimize thiopurine therapy via the increased production of 6-TGN metabolites, although, to date, the exact mechanism of this metabolic interaction remains to be determined. Considering the metabolism of thiopurines, then enzymatic inhibition or induction by allopurinol at some point in the metabolic pathway would appear to be responsible for the observed effects. Logically the inhibition of XO by allopurinol should lead to an increase in both 6-TGN and 6-MMP; however, we have demonstrated that although 6-TGN levels increased, those of 6-MMP, inexplicably, declined. Inhibition of TPMT by allopurinol would explain these results, and to this effect, we sought to show whether the addition of allopurinol to TPMT both in vitro and in vivo would lead to inhibition of the functional activity of the enzyme. In the initial in vitro experiment, increasing concentrations of allopurinol were added to an erythrocyte hemolysate, and serial TPMT assays were performed, whereas to assess the effect of allopurinol on TPMT activity in vivo, 8 healthy volunteers took allopurinol 100 mg daily for 14 days, and TPMT activity was measured before and after taking the drug. No change in TPMT activity was seen in vitro or in vivo after the addition of allopurinol (Prometheus Laboratories, San Diego, CA, unpublished data).

Another possible explanation for the observed results is simply the dose reduction of thiopurine at the time of initiation of allopurinol. Reports from the transplant literature consistently illustrate that in patients treated with AZA, TPMT activity slowly increases during treatment, presumably as a result of enzyme induction, and then declines to pretreatment levels when AZA is withdrawn. One report showed that in 23 of 26 patients commenced on AZA, TPMT activity increased by a mean of 92%.²⁰ Sixteen of 20 of our patients had the dose of 6-MP/AZA reduced to 25%–50% of the original dose, which could theoretically cause a reduction in TPMT activity and preferential shunting toward the production of 6-TGN metabolites. However, again, the biochemical response was seen equally in those patients who did or did not have doses of thiopurine reduced. However, serial functional TPMT activity measurements were not performed in our cohort.

Although we have not been able to prove any inhibitory interaction between allopurinol and TPMT, another possibility for our results would be the presence of an active, and as yet unmeasured, metabolite of allopurinol such as oxypurinol to account for the observed metabolic interaction.^{21,22} Alternatively by focusing only on metabolite levels and their metabolism in erythrocytes, we might be overlooking a potential interaction in the liver because metabolite levels in erythrocytes are reflective at least partly, and possibly substantially, of hepatic metabolism.²³ To this effect, in vitro experiments were subsequently performed to study the effect of allopurinol and its active metabolite oxypurinol on TPMT activity in both an erythrocyte hemolysate preparation and a human liver cytosol

preparation by using varying concentrations of 6-MP as the substrate. Again, no inhibition of TPMT by either allopurinol or oxypurinol was seen in either the erythrocyte or human liver cytosol preparations (Richard Weinsilboum, MD, personal communication, October, 2005). An alternative but unproven possibility might be that increased 6-TGN levels are due to induction of an enzyme involved in their production such as hypoxanthine phosphoribosyltransferase, thereby shunting substrate metabolism away from TPMT and reducing 6-MMP production, although to date there are no data to support this hypothesis. It is also possible that allopurinol itself might have beneficial effects in IBD patients independent from its effect on thiopurine metabolism, because it is known to have antioxidant effects via the inhibition of XO,²⁴ and there is in vitro evidence that it can inhibit TNF- α production and cytotoxicity from human mononuclear cells.²⁵

Potential weaknesses in our study include the relatively small sample size with the subsequent potential risk of type II statistical errors. Also, after the initial numeric metabolite data were obtained, the clinical efficacy data were then obtained retrospectively from detailed outpatient records, which might have been a source of inaccuracy, particularly with respect to measuring disease activity indices. The pHBI and partial Mayo Index are also currently not validated, although they are considered appropriate for use in outpatient clinical practice where validated indices such as the Crohn's Disease Activity Index are too cumbersome to perform routinely. In addition, serial measurements of TPMT before and after initiating allopurinol were not performed, and these could have provided valuable information as to a potential site and mechanism of action of the metabolic reaction occurring. It would also be interesting to measure the response of the individual thioguanine metabolites to the addition of allopurinol, because the clinical benefits attained by this combination suggest that it would be levels of the therapeutically active moiety, 6-TGTP, that are being increased.⁸

Another important caution regarding this therapeutic maneuver is that it does carry small possible risks that must be weighed against the potential benefits in any individual patient. In the short-term, the main risk is the induction of leukopenia, for which we advocate monitoring weekly complete blood counts for the first 4 weeks of therapy and then alternate weekly for the next 4 weeks. In this manner we were able to detect the 5 cases of leukopenia early and without clinical consequence, because all cases were reversible with thiopurine dose reduction. We did not need to use hematopoietic stimulants such as granulocyte-macrophage colony-stimulating factor to reverse leukopenia, but if these agents should be required, then this therapeutic maneuver would become less cost-effective than alternatives such as switching to methotrexate for patients with CD, although it is likely that it would still be less costly than commencing therapy with infliximab. An unlikely but potential longer-term risk is the effect of increased 6-TGN nucleotide levels on the liver, because it is known that thioguanine itself given to IBD patients can cause hepatic veno-occlusive disease or nodular regenerative hyperplasia.²⁶ However, the median levels of 6-TGN obtained in the thioguanine studies were greater than 1200 pmol/ 8×10^8 RBCs, which is much greater than the median level of 400.3 pmol/ 8×10^8 RBCs obtained in our cohort. In addition, although thioguanine-treated patients who did develop abnormal liver function tests tended to show

evidence of transaminitis, in our cohort allopurinol led to a normalization of transaminase levels. For these reasons we believe the potential for hepatotoxicity with this treatment strategy is low, but clinical follow-up of our cohort to ensure longer-term safety and efficacy is ongoing.

The use of this combination is only appropriate for CD or UC patients who preferentially metabolize mercaptopurine toward 6-MMP and are unable to enter clinical remission despite an adequate dose and duration of therapy. Eligible patients are those exhibiting increased 6-MMP production and subtherapeutic 6-TGN levels. We would recommend this maneuver be performed only when metabolite levels have been measured. Patients must also have an adequate initial WBC count to tolerate the inevitable small reduction in WBCs that occurs with the addition of allopurinol. In our cohort a WBC count of $>4.5 \times 10^8/L$ was adequate to safely initiate this therapy. This combination provides a therapeutic alternative for this subgroup of thiopurine-refractory patients who would otherwise require discontinuation of thiopurines and conversion to other agents such as methotrexate or anti-TNF therapy to achieve a corticosteroid-free remission.

In conclusion, the addition of allopurinol to thiopurine nonresponders with high TPMT activity and preferential shunting to 6-MMP metabolites successfully induces a switch toward 6-TGN metabolite production that is reflected clinically by significant improvements in disease activity scores, reduced corticosteroid requirements, and normalization of hepatic transaminase levels. Further work is required to expand cohort size, to demonstrate longer-term safety and efficacy of this maneuver, and to define a mechanism of the metabolic interaction occurring. We believe this is an important undertaking, because combination therapy has the potential to allow the sizeable subgroup of patients who preferentially metabolize thiopurines toward 6-MMP production to tolerate dose escalation, optimize 6-TGN production, and achieve the resultant clinical benefits.

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