CHAPTER 10

Allopurinol safely and effectively optimises thiopurine metabolites in autoimmune hepatitis patients

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ABSTRACT

Background
Ten percent of autoimmune hepatitis (AIH) patients are non-responsive or intolerant to thiopurine therapy. A skewed metabolism, leading to the preferential generation of (hepato)toxic thiopurine metabolites (6-MMPs) instead of the metabolic active 6-thio-guanine (thioguanine) nucleotides (6-TGNs), may explain this unfavourable outcome. Co-administration of allopurinol to low-dose thiopurine therapy may effectively revert this deviant metabolism, as has been shown in inflammatory bowel disease.

Aim
The aim of this study was to describe the effect of adding allopurinol to low-dose thiopurine therapy in AIH patients with intolerance or non-response to normal thiopurine dosages due to a skewed metabolism.

Methods
We describe the clinical efficacy and tolerability of allopurinol-thiopurine combination therapy with allopurinol 100 mg and low-dose thiopurine (25-33% of the original dosage), in eight AIH patients with a skewed thiopurine metabolism. Patients were switched because of dose limiting intolerance (n=3), non-response (n=3) or loss of response (n=2) to conventional thiopurine treatment.

Results
All eight patients showed biochemical improvement with a reduction of median alanine aminotransferase (ALT) levels of 62 U/L at start to 35 U/L at one month (p=0.03). This clinical benefit was sustained in seven patients. Allopurinol-thiopurine combination therapy effectively bypassed thiopurine side effects in four out of five patients. Median 6-thioguaninenucleotides levels increased from 100 to 200 pmol/8x10^8 red blood cells (RBC) at three months (p=0.04). Median 6-MMP levels decreased in all patients from 6090 to 175 pmol/8x10^8 RBC (p=0.01).

Conclusion
Allopurinol safely and effectively optimises thiopurine therapy in AIH patients with intolerance and/or non-response due to an unfavourable thiopurine metabolism.
BACKGROUND

Autoimmune hepatitis (AIH) is a chronic autoimmune liver disease of unknown aetiology, characterised by hypergammaglobulinemia (IgG), serum autoantibodies and histologically interface hepatitis and plasmacellular infiltrates. Current treatment strategies for AIH consist of an induction course with prednisone and frequently include subsequent addition of azathioprine (AZA) 1-2 mg/kg/day as corticosteroid-sparing maintenance therapy. Unfortunately, in 10% of AIH patients this therapeutic strategy proves ineffective, due to lack of clinical response or intolerable side effects of AZA. Before exerting its immunosuppressive potential, AZA needs to be metabolised into the pharmacologically active 6-thioguanine (thioguanine) nucleotides (6-TGN). During the complex metabolism several other (toxic) thiopurine metabolites are produced (Figure 1). The enzyme thiopurine methyltransferase (TPMT) plays a pivotal role in this process since its activity determines the level of generated methylated breakdown products, including 6-methylmercaptopurine (6-MMP). The thiopurine metabolism varies across the population, possibly as the result of different TPMT phenotypes. Consequently, the levels of 6-MMP and 6-TGN vary between individuals. High 6-MMP levels have been associated with the development of hepatotoxicity (especially elevation of transaminases) but also therapeutic failure, mainly due to concomitant lower levels of the biologically active 6-TGN. The co-administration of allopurinol alongside low-dose thiopurine redirects the thiopurine metabolism towards 6-TGN formation instead of 6-MMPs. This strategy has been successfully applied to a subgroup of inflammatory bowel disease (IBD) patients, where non-responsiveness to or side effects from thiopurine therapy were attributed to high 6-MMP levels. Here, we report the first clinical experience on efficacy and safety of allopurinol salvage therapy in AIH patients.

![Fig. 1. Azathioprine (AZA) is non-enzymatically degraded to mercaptopurine (6-MP). By several enzymatic steps (including the enzyme hypoxanthine phosphoribosyl transferase (HPRT)), 6-MP is ultimately metabolized via 6-thioinosine-monophosphate (6-TIMP) into the pharmacologically active 6-thioguaninenucleotides (6-TGN: 6-thioguanine-monophosphate (6-TGMP), 6-thioguanine-diphosphate (6-TGDP) and 6-thioguanine-triphosphate (6-TGTP)). Alternatively, 6-MP and 6-TIMP can be methylated by the enzyme TPMT leading to the formation of 6-methylmercaptopurine (6-MMP) and 6-methylthioinosine-monophosphate (6-MTIMP), respectively. Xanthine oxidase (XO) can inactivate 6-MP by the generation of 6-thiouric-acid (6-TUA).](image-url)
MATERIALS AND METHODS

Patients
Autoimmune hepatitis patients treated with allopurinol combined with thiopurines at the department of Gastroenterology and Hepatology of the VU University Medical Center (tertiary referral center) were included in this retrospective case-series analysis. Patients were switched to allopurinol-thiopurine combination therapy, if they were either non-responsive and/or were experiencing dose limiting side effects from conventional thiopurine [either AZA or mercaptopurine (MP)] therapy. Moreover, thiopurine metabolites measurements had to display preferential 6-MMP formation, arbitrarily defined as 6-MMP levels >5700 pmol/8 x 10^8 red blood cells (RBC) and/or a 6-MMP:6-TGN metabolite ratio of ≥15.

Non-response and intolerance of thiopurine therapy
Non-response and loss of response were defined as persistently raised aminotransferases (> upper limit of normal (ULN); Females: 35 IU/L; Males: 45 IU/L) despite previous response to induction therapy and thiopurine therapy, respectively. Dose limiting intolerance was defined as unbearable side effects related to the administration of thiopurines necessitating dose reduction or discontinuation of therapy.

Thiopurine metabolite measurements
Thiopurine metabolites were measured according to the method described by Dervieux-Boulieu. The 6-TGN concentrations were divided by 2,6 for comparison with the more widely used method described by Lennard. 11,12

Follow up
Patients were treated according to local protocol, receiving allopurinol 100 mg/day with a reduced dose (approximately 25-33% of original thiopurine dosage) of AZA or MP. Patients were seen at regular intervals at the outpatient clinic for clinical and laboratory parameter evaluation. Patient characteristics, reason for cessation of thiopurine monotherapy, allopurinol and thiopurine dose and duration of therapy, potential side-effects, thiopurine metabolites, biochemical and haematological parameters were recorded. In case of myelosuppression, thiopurine dosage was reduced.

Statistical analysis
Statistical analysis was performed using PASW Statistics 18 (SPSS Inc., Chicago). Demographic and therapy specific data are given descriptively. Grouped values were stated as median with range. Statistical testing between groups was done with the Mann-Whitney-U test or Wilcoxon signed rank test.
RESULTS

Baseline characteristics

Eight AIH patients (five women) with a median age of 59 years (range: 25-66 years) started with allopurinol-thiopurine combination therapy between February 2011 and October 2012 (Table 1). One patient had an AIH overlap syndrome with primary sclerosing cholangitis (PSC) and one with primary biliary cirrhosis (PBC). The median diagnostic score according to the 1999 International AIH Group criteria was 19 points (range: 7-22). The median time from the time of diagnosis prior to the switch to allopurinol-thiopurine combination therapy was 47 months (range: 17-206 months). In this time, all patients had received prednisone therapy according to standard induction tapering regime, starting with 30 mg/day. The induction therapy was followed by either AZA (n=4) or MP (n=4) therapy for a median of 50 months (range: 3-140 months) and 23 months (range: 4-33 months), respectively. Median AZA and MP doses prior to the initiation of allopurinol-thiopurine combination therapy were 125 mg AZA (range: 75-150 mg) for 2 months (range: 2-13 months) and 50 mg MP (range: 37.5-150 mg) for 19 months (range: 3-21 months), respectively (Table 1). All patients remained corticosteroid dependent with either prednisone (n=5) or budesonide (n=1) prior to the initiation of allopurinol-thiopurine combination therapy. Two additional patients (n=2) used both prednisone and budesonide for a limited period during the transition from prednisone to budesonide prior to initiation of allopurinol-thiopurine combination therapy (Table 1).

Reasons for initiation of allopurinol-thiopurine combination therapy: non-response and intolerance

Five patients were switched to allopurinol-thiopurine combination therapy after a median of 33 months (range: 3-140 months) because of non-response (n=3) or loss of response (n=2) on final conventional thiopurine dose with raised ALT levels (median: 79 U/L, range 60-111 U/L). Three patients were switched to allopurinol-thiopurine combination therapy after a median of 27 months (range: 4-56 months) due to development of one or more dose limiting side effects of AZA or MP. These included gastrointestinal complaints (nausea and vomiting; n=2), arthralgia (n=1) and headache (n=1). Two of the previously mentioned non-responders also reported persistent, but tolerable thiopurine related side effects of myalgia and arthralgia. Prior to allopurinol-thiopurine combination therapy, patients with non-response and loss of response had both higher thiopurine doses (Table 1) and metabolite levels when compared to the intolerance group [(6-TGN: 139 vs 92 pmol/8x10^8 RBC, p=0.2; 6-MMP: 7430 vs 1860 pmol/8x10^8 RBC, p=0.07)]. At the start of allopurinol treatment (100mg per day), the median daily dose of AZA (n=4) was reduced from 125 mg (range: 75-150 mg) to 25 mg (range: 25-50 mg). The median daily MP dose (n=4) was reduced from 50 mg (range: 37.5-150 mg) to 25 mg in all four patients.
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<th>Treatment</th>
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*: Time on maximum (displayed) dose of thiopurines prior to initiation of allopurinol-thiopurine combination therapy.
§: Patient 6 discontinued allopurinol-thiopurine combination therapy at 4 months because of side effects.

Abbreviations: MP, mercaptopurine; AIH, Autoimmune hepatitis; ALT, Alanine-aminotransferase; AZA, Azathioprine; B, budesonide; CT, allopurinol-thiopurine combination therapy; IAIHG, international AIH group diagnostic score; F, female; M, male; N, no; P, prednisone; PBC, primary biliary cirrhosis, PSC, primary sclerosing cholangitis; Y, yes
Clinical and biochemical effectiveness

The median follow up after initiation of allopurinol-thiopurine combination therapy was 13 months (range: 7-18 months). The treatment regimen was clinically effective in all but one patient with improvement of median baseline ALT levels from 62 U/L (range: 26-111 U/L) to 35 U/L (range: 26-48 U/L) at 1 month (p=0.03), 24 U/L (range: 26-48 U/L) at three months (p=0.08) and 29 U/L (range: 19-112 U/L) at six months (p=0.03; Figure 2). Four out of five non-responders did sustain the biochemical improvement during follow up. One patient developed raised aminotransferases at three months (ALT 122 U/L) after initial improvement at 1 month (ALT: 48 U/L). Despite this, allopurinol-thiopurine combination therapy was continued in this patient, showing spontaneous biochemical improvement (ALT 41 U/L) at 6 months. The group of AIH patients (n=3) that started allopurinol-thiopurine combination therapy because of intolerance, reported disappearance of side effects and had disease follow up without incidents. After initiation of allopurinol-thiopurine combination therapy, the prednisone dosages could be lowered in five patients whereas in the other patients the steroid dosages remained unchanged. No flares of AIH occurred during follow-up necessitating dose escalation of steroids.

![Figure 2](image-url)  
Fig. 2. Overall reduction in median ALT levels (U/L) during 6 months of allopurinol-thiopurine combination therapy.

Tolerability and adverse events during allopurinol-thiopurine combination therapy

Overall, the treatment regimen was well tolerated in all but two patients. One prior non-responder, with concomitant multiple sclerosis (MS), reported progressive myalgia and worsening complaints of pre-existent hyperesthesia and numbness of hands and feet four months after commencement of combination therapy. She associated these com-
plaints with the novel treatment regimen and stopped allopurinol-thiopurine combination therapy and started taking MP in the original dosage (100 mg/day). Subsequently her complaints of neuropathy improved. Overall white blood cell counts (WBC; normal range: 4–10 x 10⁹/L) showed a mild decrease after initiation of allopurinol-thiopurine combination therapy from 6.3x10⁹/L (range: 3.6-9.0x10⁹/L) to 4.9x10⁹/L (range: 4.4-7.6 x10⁹/L) at six months (p=0.2). One patient developed a mild pancytopenia [haemoglobin: 7.1 mmol/L (normal range: 8.5–11.0 mmol/L), white blood cell count: 2.9 x 10⁹/L and thrombocytes: 145 x 10⁹/L (normal range: 150–400 x 10⁹/L)] at 13 months of therapy with 6-TGN levels of 196 pmol/8x10⁸ RBC. It should be noted that this patient was also using hydroxychloroquine as treatment for Sjögren's disease. As blood cells counts remained stable during 4 months of subsequent follow up, he continued allopurinol-thiopurine combination therapy without dose adjustment of AZA (75 mg/day) and allopurinol (100 mg/day).

**Thiopurine metabolites levels**

Median levels of 6-TGN increased from 100 pmol/8x10⁸ RBC (range: 50-185 pmol/8x10⁸ RBC) at baseline to 200 pmol/8x10⁸ RBC (range: 54-265 pmol/8x10⁸ RBC) at three months (p=0.04)(Figure 3). Simultaneously, levels of 6-MMP decreased from a median of 6090 pmol/8x10⁸ RBC (range: 1700-9000 pmol/8x10⁸ RBC) at baseline to 175 pmol/8x10⁸ RBC (range: 0-490 pmol/8x10⁸ RBC) at one month (p=0.01)(Figure 4). This steep decrease was observed in all patients (Figure 4). The observed median 6-MMP/6-TGN ratio decreased in all patients from 55.7 (range: 18-122) at baseline to 1.3 (range: 0-2) during allopurinol-thiopurine combination therapy (p=0.01).

![Fig. 3. 6-Thioguaninenucleotide levels (pmol/8x10⁸ RBC) before and at 12 weeks of allopurinol-thiopurine combination therapy.](image-url)
**DISCUSSION**

**Clinical and biochemical effectiveness**

In this study we demonstrate that AIH patients suffering from ineffectiveness or intolerance to AZA or MP due to an unfavourable thiopurine metabolism, the combination therapy of low-dose thiopurine combined with allopurinol proved an effective and well tolerated alternative immnosuppressive maintenance strategy. Despite the heterogeneity and small size of the studied patient group, regarding to type and duration of prior thiopurine therapy, the clinical benefit was marked by a sustained reduction and normalisation of ALT levels in seven out of eight patients. The majority of patients with previous intolerable adverse events during initial thiopurine therapy were able to tolerate allopurinol-thiopurine combination therapy without development of major drug-related side-effects. These observations are consistent with reports on allopurinol-thiopurine combination therapy in IBD patients displaying (non-)hepatotoxic adverse events.9

**Adverse events**

Two patients developed mild adverse reactions, being neuropathy and myelodepression, during allopurinol-thiopurine combination therapy. Reversible peripheral neuropathy is a rarely reported side-effect of allopurinol and although in our patient these complaints may also be attributed to disease activity of MS, this led to early cessation of allopurinol-thiopurine combination therapy.14 Development of uncomplicated, mild pancytopenia
was noted in the second patient after 15 months of follow up. Despite the overall mild decrease in WBC at 6 months, this case illustrates the need for continued monitoring of blood cell counts in all patients during thiopurine therapy.

**Changes in thiopurine metabolites levels**

The co-administration of allopurinol in these AIH patients with a deviant thiopurine metabolism led to a steep decrease in 6-MMP levels and a mild increase in 6-TGN levels. A 6-TGN level of 220 pmol/8x10^8 RBC has been reported as the optimal cut-off value as predictor for remission in AIH (sensitivity: 83% specificity: 62%).15 Although most of our patients did not reach this threshold, the observed promising clinical outcome after switching to allopurinol-thiopurine combination therapy underlines reported associations between inefficacy, toxicity and elevated 6-MMP levels combined with low 6-TGN levels.15 The pharmacological explanation of this marked thiopurine metabolism modulation after co-administration of allopurinol is still enigmatic, as the activity of the methylating enzyme TPMT appears to be unaffected by allopurinol.7

**Implications for second line therapy**

Currently, there is no established second line maintenance therapy for AIH. Several alternative drugs, such as mycophenolate mofetil (MMF), tacrolimus or cyclosporine, have been studied in small series of patients.16 Although showing good results (70% remission) in treatment naïve AIH patients, administration of MMF as salvage therapy led to an improvement of reported side-effects in patients with dose limiting intolerance to AZA but it was considerably less effective in non-responders.17-19 Ciclosporine and tacrolimus both are calcineurin inhibitors which have primarily been studied as remission induction agents in AIH patients with lack of response or intolerance to prednisone.16 Recently, it has also been suggested that calcineurin inhibitors might be effective as long-term alternative to thiopurines in cases of refractory disease.2 Yet, in thiopurine refractory patients with preferential 6-MMP metabolism, thiopurine-allopurinol combination therapy might prove to be beneficial over the more costly and (nephro-)toxic calcineurin inhibitors.20,21

In conclusion, allopurinol in combination with low-dose thiopurine might be an effective and relatively safe alternative immunosuppressive strategy for AIH patients failing standard thiopurine therapy due to preferential 6-MMP metabolism. The present report is limited by its small and heterogeneous patient group and therefore larger and controlled studies are needed to confirm the promising outcomes of this combination therapy.
REFERENCES


