PHARMACOKINETICS AND DISPOSITION

Low allopurinol doses are sufficient to optimize azathioprine therapy in inflammatory bowel disease patients with inadequate thiopurine metabolite concentrations

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Abstract

Purpose Recent studies in patients with inflammatory bowel diseases (IBD) on thiopurine therapy suggest that too low 6-thioguanine nucleotide concentrations (6-TGN) and too high methylmercaptopurine nucleotide concentrations (MMPN) can be reversed by a combination therapy of allopurinol and low-dose thiopurines. To date, however, optimal dosing has not been established. The aim of this study was to evaluate the minimal allopurinol doses necessary to achieve adequate 6-TGN concentrations in combination with low-dose azathioprine.

Methods A stepwise dose-escalation of allopurinol was performed in 11 azathioprine-pretreated IBD patients with inadequately low 6-TGN concentrations (<235 pmol/8×10^8 erythrocytes) and/or elevated MMPN concentrations (>5,000 pmol/8×10^8 erythrocytes) and/or elevated liver enzymes (alanine aminotransferase and/or aspartate aminotransferase levels one- to threefold the upper limit of normal). Six patients were recruited into an open study, and five were treated in the context of an individualized therapeutic approach. Adverse effects, azathioprine metabolites, liver enzymes and whole blood counts were monitored two to three times per month.

Results Adequate 6-TGN concentrations were achieved with a combination of 25 mg allopurinol and 50 mg azathioprine in one patient and with 50 mg allopurinol and 50 mg azathioprine in nine patients. Median 6-TGN concentrations (range) were 336 (290–488) pmol/8×10^8 erythrocytes after an 8-week-long intake of the final dose combination. One patient dropped out due to nausea after the first intake. MMPN concentrations and liver enzymes normalized immediately in all affected patients. All patients finishing the dose-escalation regimen tolerated the treatment without toxicity.

Conclusions Combination therapy with only 50 mg allopurinol and 50 mg azathioprine daily is sufficient, efficacious and safe in most IBD patients with inadequate thiopurine metabolite concentrations to optimize azathioprine-based IBD therapy.

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Introduction

In patients with inflammatory bowel disease (IBD), the thiopurine immunomodulators azathioprine and 6-mercaptopurine are used to induce and maintain remission. These substances are prodrugs that undergo a complex metabolic transformation resulting in the formation of the pharmacologically active, immunosuppressive metabolites 6-thioguanine nucleotides (6-TGN) [1]. In a competing pathway, the formation of methyl-thiopurines, such as the methylmercaptopurine ribonucleotides (6-MMPN), which are not directly immunosuppressive, is catalyzed by the enzyme thiopurine methyl transferase (TPMT). Between 10 and 15 % of IBD patients are thiopurine refractory due to the preferential production of 6-MMPN rather than active 6-TGN [1–4]. This may result in insufficient clinical response and/or an increased rate of adverse effects [2, 5]. Upon dose-escalation in these patients, concentrations of 6-TGN often remain below the lower limit of the therapeutic range of approximately 235 pmol/8×10^8 erythrocytes (EC) [2] while conversely 6-MMPN concentrations increase above a value of 5,000 pmol/8×10^8 EC, which has been associated with an increased risk for hepatotoxicity [1]. Several studies have recently provided evidence that IBD patients with an unfavorable MMPN/6-TGN ratio can benefit from adding allopurinol to a reduced dose of thiopurines. The effects of this combination therapy are (1) an increased production of 6-TGN, (2) a reduced formation of 6-MMPN, (3) a normalization of liver function tests and (4) better disease control and less corticosteroid usage [6–12]. In terms of the metabolic pathway of thiopurines, the shunting of metabolism towards the production of 6-TGN induced by allopurinol can be explained by inhibition of the enzyme xanthine oxidase. However, the mechanism by which allopurinol leads to a reduction in 6-MMPN concentrations has not yet been determined.

In previous studies and case reports, thiopurine doses were reduced to 25–50 % of the initial thiopurine dose and usually combined with daily doses of allopurinol 100–200 mg [6–9, 12–15] or even 300 mg [10]. The thiopurine dose was reduced empirically or according to published recommendations. Accordingly, in their study, Ansari et al. [9] reduced the thiopurine dose to 25–33 % of the monotherapy dose as proposed earlier for the use of thiopurine in renal transplant patients [16]. Other researchers [15] reduced the thiopurine dose to 25 % of the standard dose based on the report by Sparrow et al. [6]. However, the rationale for the selection of daily doses of 100–200 mg allopurinol has never been provided; this is particularly true in the case of the combination therapy in renal transplant recipients, where only 25 mg allopurinol each second day was administered [16]. To our knowledge, the optimal allopurinol–azathioprine combination dose has

not been studied systematically in IBD patients, and neither the minimally effective nor the maximally tolerated doses of this combination are as yet known. The appropriate dose adjustment with a decrease of both azathioprine and allopurinol drug exposures to a minimum may help to avoid toxic side effects. Accordingly, we conducted a dose-escalation study to evaluate the minimal allopurinol dose that, in combination with low-dose azathioprine, would result in therapeutic 6-TGN concentrations in azathioprine-pretreated patients. Our secondary objectives were to evaluate whether maintenance of remission could be achieved with the low dose allopurinol–azathioprine combination and whether allopurinol influences TPMT activity.

Materials and methods

Study procedures

We conducted a prospective, open, non-randomized single-center cohort study in patients with IBD. Six otherwise healthy female azathioprine-pretreated patients with Crohn's disease (CD) and ulcerative colitis (UC) who had received subtherapeutic 6-TGN concentrations (<235 pmol/8×10^8 EC) and insufficient disease control despite adequate therapy dosing and duration, and/or had elevated 6-MMPN concentrations (>5,000 pmol/8×10^8 EC) and/or elevated liver enzymes [alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) of one- to threefold the upper limit of normal range (ULN)] were included in the study. Additionally, we performed a stepwise dose-escalation of allopurinol in five male and female CD and UC patients who did not meet the study eligibility criteria (e.g. patients with relevant comorbidities or obesity), but who we considered would benefit clinically from the combination therapy of allopurinol and low-dose azathioprine. These patients were treated with allopurinol in combination with azathioprine in the context of an individualized therapeutic approach.

The inclusion criteria were: age ≥18 years, normal weight with a body mass index (BMI) of 18–30 kg/m^2, a confirmed diagnosis of either CD or UC based on clinical, endoscopic and histologic criteria, normal TPMT activity (≥30 nmol MTG/gHb/h), azathioprine metabolite concentrations and/or hepatic azathioprine side effects as defined above. The main exclusion criteria were pregnancy or lactation, any current or previous immunosuppressive therapy except for corticosteroids and/or azathioprine within 3 months before the first study-drug intake and any clinically relevant comorbidity (including malignancy and relevant infections in the last 3 months), as based on extensive medical history, physical examination, vital signs, routine laboratory screen and 12-lead electrocardiogram (ECG).

On day 1 of the study, the azathioprine dose was reduced to a dose of 50 mg once daily (qd) for one 28-day therapy cycle. Allopurinol 25 mg qd was added to the therapeutic regimen on day 1 of the second cycle, if the 6-TGN concentrations were <350 pmol/8×10^8 EC and if no relevant toxicity had occurred (Fig. 1). A subsequent cycle with a higher dose of either allopurinol or azathioprine according to the dose escalation scheme (Fig. 1) was started if the two aforementioned criteria were not met, otherwise, the follow-up month was begun. In the case of severe toxicity, the combination was stopped and the patient excluded from the study. A total of seven cycles and 1 month of follow-up examination was the maximum scheduled study duration. The study patients received commercially available azathioprine 50 mg film-coated tablets (Imurek®; Glaxo SmithKline, Münchenbuchsee, Switzerland, and ProConcepta Zug, Zug, Switzerland), while allopurinol 25 mg and 50 mg capsules were manufactured by the Cantonal Pharmacy of Zürich, Zürich, Switzerland.

The following study procedures were carried out repeatedly in each patient: update of medical history, determination of adverse events, measurement of body temperature and laboratory measurements three times per cycle (on study days 8–10, 23–25 and 26–28) and a short physical examination. Crohn's Disease Activity Index (CDAI)/Rachmilewitz disease activity scores were evaluated to assess clinical response. Repeated laboratory measurements consisted of a complete blood cell count, creatinine, lactate dehydrogenase, AST, ALT, alkaline phosphatase, gamma-glutamyltransferase, total bilirubin, C-reactive protein and concentrations of the thiopurine metabolites 6-TGN and 6-MMPN in serum and blood, respectively. The TPMT activity was measured at screening and once every month thereafter because the effect of allopurinol on TPMT activity was not known at the time of study design. At the end of each cycle, the allopurinol dose was adapted according to tolerability, clinical response, hematology and clinical chemistry parameters and 6-TGN concentrations.

The dose-escalation was finished in an individual patient when 6-TGN concentrations were ≥350 pmol/8×10^8 EC or if moderate/serious toxicity occurred (Fig. 1). The study ended for each patient after 1 month of follow-up with an end-of-study examination that consisted of laboratory measurements, physical examination, 12-lead ECG and CDAI/Rachmilewitz index assessment.

For the patients who were treated with the azathioprine–allopurinol combination in the context of an individualized therapeutic approach, dose increments were approximated to the study protocol. The azathioprine dose was initially reduced to 50 mg qd for 2–3 weeks, following which an allopurinol dose of 50 mg qd was added. Further monthly allopurinol dose increases in steps of 25 mg were intended in case of persisting subtherapeutic 6-TGN concentrations. Determination of adverse effects and metabolite and other laboratory measurements were performed twice each month, and physical examinations were performed at least once each month. The patients took in commercially available azathioprine film-coated tablets (Imurek®) and commercially available allopurinol tablets (either Mephanol® 100 mg, Mepha Pharma, Aesch BL, Switzerland, or Allopur® 100 mg, Sandoz, Cham, Switzerland), and each patient was responsible for dividing the tablets into the proper dose.

The study was approved by the Ethics Committee of the Canton of Zurich, Switzerland and by the Swiss Health Authority Swissmedic, Bern, Switzerland. It has been registered at ClinicalTrials.gov with the number NCT00849368.

Metabolite measurements

The cycle duration of 28 days was chosen to ensure that metabolite concentrations reflected steady state before a dose increase was performed, as the average half-life of 6-TGN in EC is 5 days [17–19].

Blood samples for the measurement of 6-TGN and 6-MMPN concentrations were collected at least 5 h after the daily drug intake. Blood was collected in vacutainers supplemented with heparin (5 ml per sample), then spiked with 100 μl of the antioxidant 1,4-dithio-D,L-threitol at a concentration of 1 mg/ml and stored at −20 °C until analysis. 6-TGN and 6-MMPN were measured using a validated, specific and selective high-performance liquid chromatography (HPLC) method [20, 21]. The HPLC system consisted of a 9010 pump, a ProStar 410 autosampler and a 9050 UV-VIS detector (Varian, Sunnyvale, CA). The lower limit of quantification (LLQ) was 50 pmol/8×10^8 EC for TGN and 100 pmol/8×10^8 EC for MMPN. For values under the LLQ, one-half of the LLQ was considered for further analysis.

TPMT activity

The TPMT assay (5 ml EDTA whole blood) was performed according to Kröplin et al. [22] with minor modifications. The Hb of the red blood cell lysate was measured by a Microcell counter F500 (Sysmex, Hamburg, Germany). The sample volume of each patient consisted of 50 μl red blood cell lysate (in accordance with the published method), whereas the extraction of the product was made with dichlormethane/2-propanol (90/10; v/v). The same HPLC system as for metabolite quantifications was used. The cut-off between normal and intermediate or low TPMT activity was defined as 30 nmol MTG/gHb/h.

Statistical analysis

Descriptive analyses were performed in all 12 patients. The results are presented as median values and ranges.
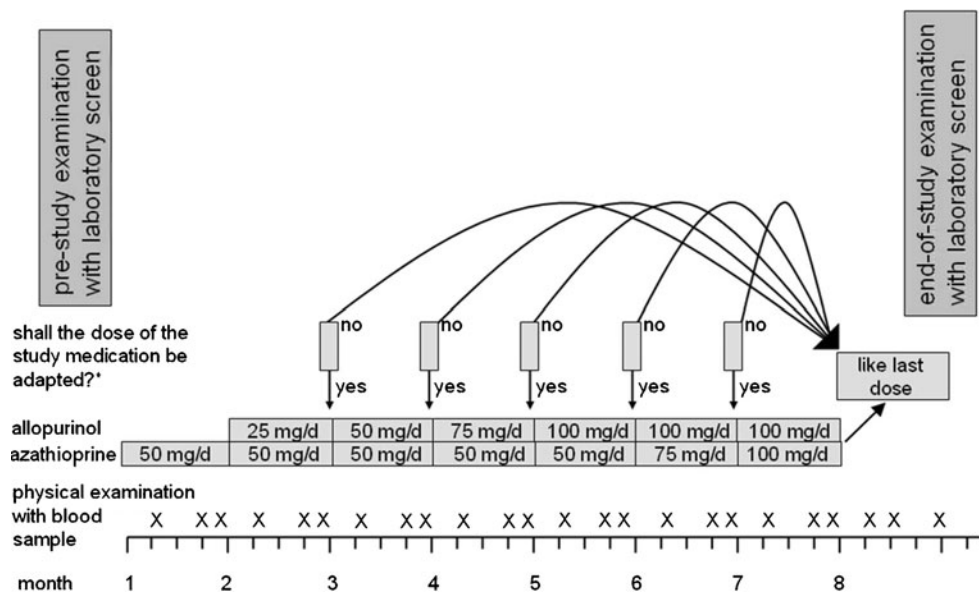
Fig. 1 Study dose escalation scheme. *Asterisk* If 6-thioguanine nucleotide (6-TGN) concentrations were <350 pmol/8×10^8erythrocytes (EC) and there were no signs of major or minor toxicity, the next dose increment was initiated. Serious toxicity was defined as: neutrophils <500/μl and/or hemoglobulin (Hb) decline of >2 g/dl and/or thrombocytes <100,000/μl and/or lymphocytes <500/μl and/or rise in alanine/aspartate aminotransferases (ALT/AST) of >50 % of the pre-study examination value and/or diagnosis of pancreatitis independent of 6-TGN concentrations. Moderate toxicity was defined as: neutrophils 1,000–1,500/μl, Hb decline of <2 g/dl, thrombocytes 100,000–140,000/μl, ALT/AST rise of 15–50 % of the pre-study examination. In case of moderate toxicity: maintenance of dose level, in case of persistence for >1 month: study termination

Results

Study patients

The baseline characteristics of the study patients are presented in Table 1.

At inclusion, five of six patients had 6-TGN concentrations below the recommended threshold of 235 pmol/8×10^8EC (Fig. 2a) and five of six patients showed elevated MMPN concentrations of >5,000 pmol/8×10^8 EC (Fig. 3a). One of these patients initially had normal 6-TGN. Four patients needed corticosteroids at study entry. Three patients had mildly elevated liver transaminases up to threefold the ULN (all considered to be azathioprine-related), one had an additional total bilirubin elevation (1.5-fold ULN). With the exception of slightly reduced neutrophil counts in one patient (1,220/μl, normal range 1,400–8,000/μl) and reduced lymphocyte counts in three patients (270–970/μl, normal range 1,500–4,000/μl), all other laboratory values, including markers of inflammation, were normal.

In one of the two patients with CD, the CDAI of 162 points revealed clinically active disease (active disease >150 points). The other CD patient (CDAI 40 points) and all four patients with UC were in clinical remission (Rachmilewitz index <4 points).

Five of six patients completed the study. One patient achieved therapeutic 6-TGN concentrations with a combination of allopurinol 25 mg qd and azathioprine 50 mg qd, whereas four patients needed allopurinol 50 mg qd in combination with azathioprine 50 mg qd (Fig. 2a). One CD patient dropped out due to nausea at the azathioprine 50 mg dose level. The median 6-TGN and MMPN concentrations, TPMT activity, leukocyte and lymphocyte counts and ALT, AST and bilirubin values at the different azathioprine and allopurinol dose levels are shown in Table 2. In all five patients who completed the study, MMPN concentrations normalized during the first 8–10 days after the reduction of azathioprine to 50 mg qd (first cycle) and remained normal after the addition of allopurinol to the therapeutic regimen (Fig. 3a). In both patients with initially elevated liver enzymes who completed the study, liver enzymes normalized at the azathioprine 50 mg qd dose level and remained stable thereafter. One patient with initially normal liver enzymes developed elevated transaminases up to 5.7-fold greater than the ULN in the course, which normalized at follow-up without any modification of the doses of azathioprine and allopurinol. At the end of the study laboratory examinations revealed normal liver enzyme levels in all patients. Lymphocytes were below the lower limit of normal range in three patients 1,190–1,300/μl at the end of the follow-up. Routine laboratory testing found no relevant abnormalities in any patient at the end of the study.

The CDAI values and the Rachmilewitz indices were below 150 and 3, respectively, throughout the study in four patients. The administration of corticosteroids was stopped in three patients after the addition of allopurinol. One UC patient with azathioprine and rectal corticosteroids at study

Table 1 Baseline characteristics of the study patients

Baseline characteristics	Values[a] (range)
Median age (years)	40.5 (23–47)
Gender (female/male) (n)	6 / 0
Diagnosis (n)	
Crohn's disease	2
Ulcerative colitis	4
Median disease duration (years)	4.5 (0.5–20)
Crohn's disease activity index (CDAI)	40–162
Rachmilewitz Index for ulcerative colitis	1–3
Median body mass index (kg/m²)	24.7 (20.4–37.5)
Concomitant medications (n)	
ASA	3
Calcium	5
Vitamin D3	6
Corticosteroids (oral)	2
Corticosteroids (rectal)	3
Median AZA[a] dose prior to study entry (mg/day)	150 (125–200)
Median AZA[a] dose prior to study entry (mg/kg bw/day)	2.2 (2.0–2.9)
Median 6-TGN concentrations (pmol/8×10^8EC)	208 (106–270)
Median MMPN concentrations (pmol/8×10^8EC)	7,678.5 (2,351–16,928)
Median TPMT activity (nmol MTG/gHb/h)	44.5 (34–57)
Median leukocytes (/μl)	6,680 (2,390–8,120)
Median lymphocytes (/μl)	1,240 (270–2,400)
Median total bilirubin (μmol/l)	10 (8–31)
Median ALT (U/l)	35.5 (15–105)
Median AST (U/l)	29.5 (21–45)

ASA, 5-aminosalicylic acid; AZA, azathioprine; bw, body weight; 6-TGN, 6-thioguanine nucleotide; MMPN, methylmercaptopurine nucleotide; TPMT, thiopurine methyl transferase; ALT/AST alanine/aspartate aminotransferase

[a] Normal ranges: Leukocytes, 3,000–9,600 /μl; lymphocytes, 1,500–4,000 /μl; bilirubin, <21 μmol/l; ALT, 10–35 U/l (female)/10–50 U/l (male); AST, <35 U/l (female)/<50 U/l (male)

entry, who was in remission based on clinical and endoscopic evidence, relapsed at the azathioprine 50 mg dose level (first cycle) and exhibited an intermittent liver enzyme increase. The therapeutic regimen of this patient was supplemented with oral prednisone until the follow-up period. The Rachmilewitz indices in this patient were 3, 11, 1, 4, 4 and 0.

At the end of the study all patients were clinically in remission and were receiving neither oral nor rectal corticosteroids. With the exception of the above-mentioned transient liver enzyme increase, all patients who finished the study tolerated the azathioprine–allopurinol combination without relevant signs of toxicity.

TPMT activity

Thiopurine methyl transferase activity was normal in all patients at study entry and did not change after the addition of allopurinol to the therapeutic regimen (Table 2).

Non-study patients

The baseline characteristics of the five IBD patients who were treated with azathioprine and allopurinol in the context of an individualized therapeutic approach are presented in Table 3. Concomitant diseases precluding study inclusion were a chronic depression, bronchial asthma, obesity (BMI 35 kg/m²), hypertensive and coronary heart disease and primary sclerosing cholangitis. At the time of allopurinol initiation, two patients were receiving oral corticosteroids. All patients had subtherapeutic 6-TGN concentrations ranging from 71 to 234 pmol/8×10^8 EC (Fig. 2b) and three had elevated MMPN concentrations of >5,000 pmol/8×10^8 EC (Fig. 3b). The median 6-TGN and MMPN concentrations, leukocyte and lymphocyte counts and ALT, AST and bilirubin values at the different azathioprine and allopurinol dose levels are shown in Table 4. All patients had active disease based on clinical and/or endoscopic evidence despite adequate azathioprine dosing of 2–2.5 mg/kg body weight and adequate therapy duration. In one CD patient infliximab as an add-on therapy to oral budesonide and azathioprine was stopped 5 months before initiation of the azathioprine–allopurinol combination therapy due to intolerable pruritus and erythema. Following discontinuation of infliximab the patient suffered from clinically active disease despite therapy with 9 mg oral budesonide qd in combination with azathioprine. The other CD patient had persistent abdominal pain and elevated calprotectin levels in the feces. One UC patient had recurrent bloody diarrhoea, persistently elevated calprotectin values in the feces and chronic iron deficiency, all of which are indicators of chronic active disease. One UC patient with pancolitis showed low 6-TGN concentrations and chronic active disease based on endoscopic findings. This patient refused tumor necrosis factor (TNF) blocker therapy. The third patient with UC had endoscopically confirmed moderate left-sided colitis and needed oral prednisone repeatedly.

All patients had normal TPMT activities in the beginning and during the dose-escalation procedure. Total bilirubin was

Fig. 2 Individual 6-TGN concentration vs. time courses in five patients participating in the dose-finding study (**a**) and in five patients who received the combination outside the study (**b**). **a** Patients received 50 mg azathioprine daily (median 0.7 mg/kg, range 0.6–0.9 mg/kg) as monotherapy for 28 days before the combination was started. *Dotted lines* Therapeutic ranges, *dashed line* study target concentration. **b** Patients received 50 mg azathioprine daily (median 0.7 mg/kg, range 0.5–1.0 mg/kg) as monotherapy for 2–3 weeks, before the combination therapy was started. *Dotted line* Minimal recommended 6-TGN concentration [235 pmol/8×10^8 erythrocytes (*EC*)]
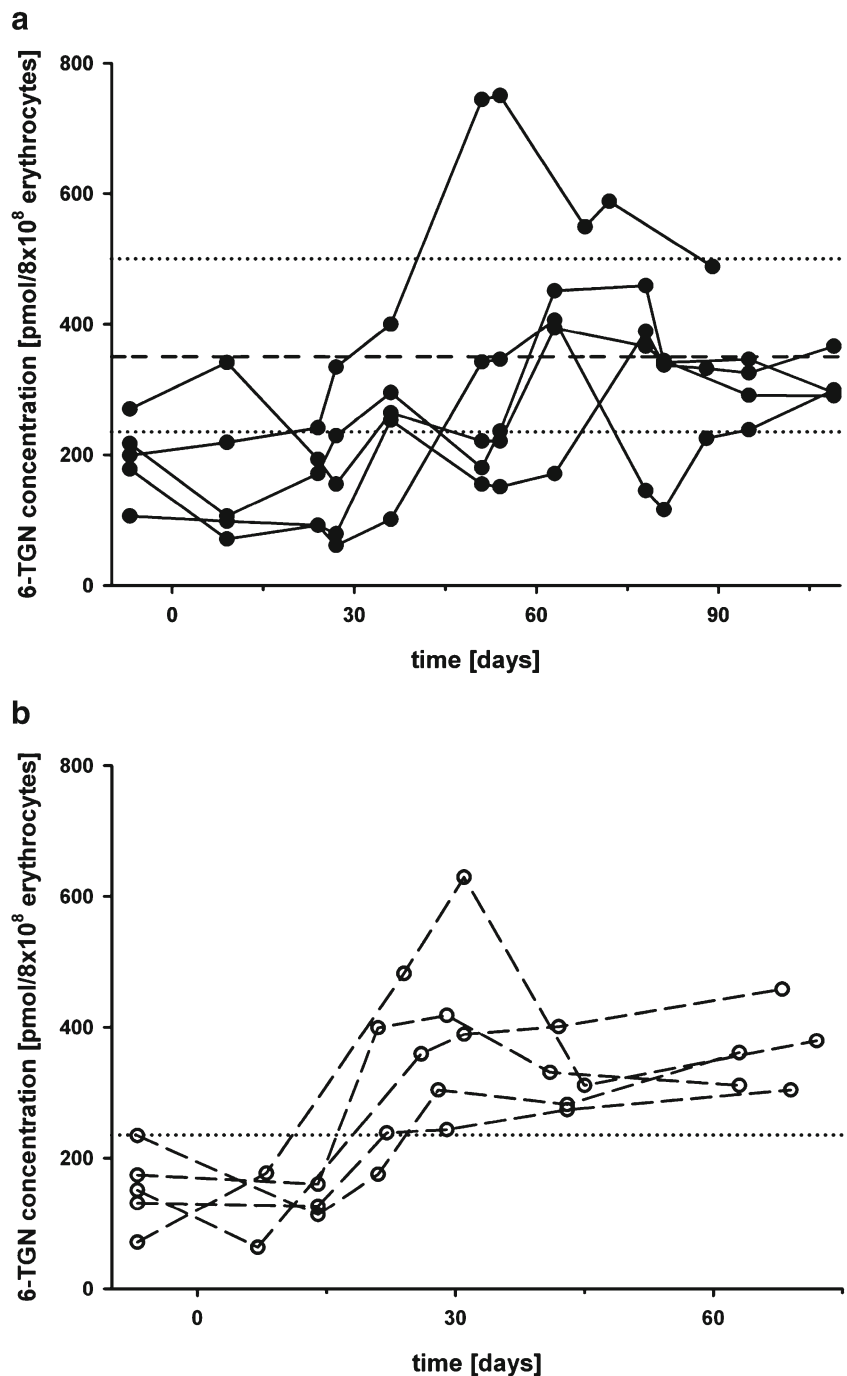
elevated in one patient with UC who had a genotypically confirmed Gilbert's syndrome [homozygous mutation in the UGT1A1 promoter, genotype (TA)7/(TA)7]. The other four patients had normal liver enzymes. Routine laboratory examinations detected no pathological findings with the exception of moderate lymphopenia in four patients (750–1,250/μl, normal range 1,500–4,000/μl), mild anemia in two patients (12.6 g/dl, normal range 13.4–17.0 g/dl), mild leukopenia in one patient (2,850/μl, normal range 3,000–9,600/μl) and elevated C-reactive protein in one patient (6.3 mg/l, reference <5 mg/l).

All patients achieved stable therapeutic 6-TGN concentrations with azathioprine 50 mg qd and allopurinol 50 mg qd (Fig. 2b). In all patients MMPN concentrations dropped to normal values ranging between 228 and 1,581 pmol/8×10^8 EC after the azathioprine dose had been reduced to 50 mg qd. MMPN concentrations remained low or dropped further in all patients during dose-escalation and follow-up (Fig. 3b). Liver enzymes remained normal in all patients. At the end of the follow-up no patient had leukopenia or neutropenia. Except for stable lymphopenia, other significant laboratory abnormalities were not observed.

Fig. 3 Individual methylmercaptopurine nucleotide (*MMPN*) concentration vs. time courses in five patients participating in the dose-finding study (**a**) and in five patients who received the combination outside the study (**b**). **a** Patients received 50 mg azathioprine daily as monotherapy for 28 days, before the combination was started. **b** Patients received 50 mg azathioprine daily as monotherapy for 2–3 weeks, before the combination was started. *Dotted line* Upper limit of normal MMPN concentrations (5,000 pmol/8× 10^8 EC)
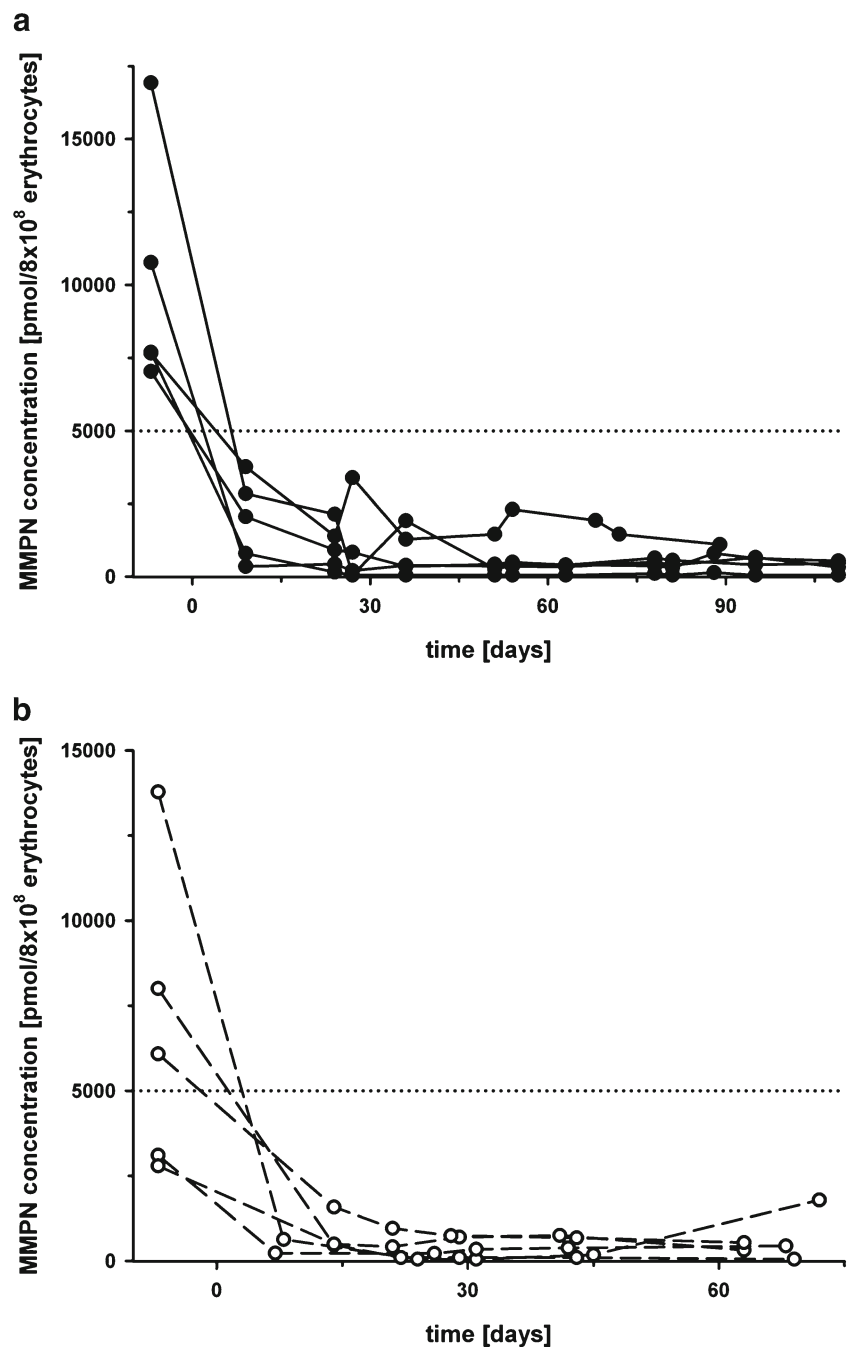
At the end of the follow-up, all three non-study UC patients were in remission (two patients clinically and the patient with initially chronic-active pancolitis, both clinically and endoscopically). One CD patient was clinically in remission with intake of a reduced budesonide dose. One CD patient improved clinically, but still had very mild ileitis based on endoscopic evidence. Corticosteroid treatment had ceased in one of two patients with initial intake and had tapered in the other one. The allopurinol–azathioprine combination therapy was well tolerated by all patients.

Long-term follow up

Although the scheduled follow-up according to the protocol was only 1 month, we can report a much longer follow-up for several patients. After 3 years of combination therapy, one non-study UC patient and one study CD patient are still in remission without signs of toxicity. After 1 year of therapy, one UC patient is in remission based on clinical and endoscopic findings. Another UC patient relapsed 6 months after study completion and finally required TNF-alpha blocker therapy. The follow-up for the other study and non-

Table 2 Results of the laboratory examinations of the study patients at the different dose levels

Laboratory parameter	Azathioprine 50 mg/day			Azathioprine 50 mg/day + allopurinol 25 mg/day			azathioprine 50 mg/day + allopurinol 50 mg/day			Maintenance dose		
	Days 8–10	Days 23–25	Days 26–28	Days 8–10	Days 23–25	Days 26–28	Days 8–10	Days 23–25	Days 26–28[a]	Follow-up week 1[b]	Follow-up week 2	Follow-up week 4
6-TGN (pmol/ 8×10^{8}EC)	106 (71–341)	171 (92–241)	155 (61–334)	264 (101–400)	221 (155–744)	236 (151–750)	400 (171–451)	377.5 (145–459)	339 (116–344)	332 (225–549)	325 (238–588)	299 (290–488)
MMPN (pmol/ 8×10^{8}EC)	2,060 (350–3,768)	917 (156–2,141)	206 (50–3,391)	387 (50–1,921)	366 (50–1,282)	367 (50–2,304)	368 (50–404)	441 (113–639)	352 (50–561)	804 (141–1,931)	630 (50–1,455)	455 (50–1,106)
TPMT activity (nmol MTG/ gHb/h)			45 (35–65)			44 (41–52)			51 (41–62)			46 (32–66)
Leukocytes (/µl)			8,160 (3,700–11,130)			7,600 (5,170–16,390)			5,300 (3,700–8,830)			6,230 (4,480–10,520)
Lymphocytes (/µl)			1,700 (780–3,140)			1,140 (1,030–2,760)			1,810 (810–2,550)			1,300 (1,190–2,490)
Bilirubin total (µmol/l)			7 (5–24)			8 (7–27)			10.5 (6–12)			10 (6–15)
ALT (U/l)			23 (16–51)			25 (11–116)			18.5 (10–124)			18 (7–26)
AST (U/l)			24 (21–29)			21 (15–49)			23 (16–45)			22 (14–27)

Normal ranges and abbreviations: See footnote to Table 1

Data are presented as the median (n = 5) with the range in parenthesis

[a] Only 4 patients in this dose level

[b] Values for two patients are missing due to stay abroad

Table 3 Baseline characteristics of the non-study patients

Baseline characteristics	Values (range)
Median age (years)	35 (20–68)
Gender (female/male) (n)	2/3
Diagnosis (n)	
Crohn's disease	2
Ulcerative colitis	3
Median disease duration (years)	9 (2–15)
Median body mass index (kg/m^2)	24.8 (17.9–35.0)
Concomitant medications (n)	
ASA	3
Calcium	1
Vitamin D3	1
Corticosteroids (oral)	1
Corticosteroid s(rectal)	3
Antihypertensives	1
Statins	1
Thyroid hormone	1
Antiplatelets	1
Antidiarrheals	2
Spasmolytic drugs/analgesics	2
Antipsychotics	1
Benzodiazepines	1
Antiasthmatics	1
Colestyramine	1
Other vitamins	1
Median AZA dose prior to study entry (mg/day)	150 (100–250)
Median AZA dose prior to study entry (mg/kg bw/day)	2.2 (1.8–2.3)
Median 6-TGN concentrations (pmol/8×10^{8}EC)	151 (71–234)
Median MMPN concentration (pmol/8×10^{8}EC)	6,086 (2,794–13,783)
Median TPMT activity (nmol MTG/gHb/h)[a]	52.5 (44–56)
Median leukocytes (/µl)	7,250 (2,850–8,910)
Median lymphocytes (/µl)	1,150 (750–2,040)
Median total bilirubin (µmol/l)[b]	7 (3–25)
Median ALT (U/l)	24 (12–50)
Median AST (U/l)	30 (22–34)

Normal ranges and abbreviations: See footnote to Table 1

[a] Value of one patient, which was assessed after the addition of allopurinol to the therapeutic regimen, was not included

[b] The patient with an elevated total bilirubin had confirmed Gilbert's syndrome with a homozygous mutation in the UGT1A1 promoter [UGT1A1 promoter genotype (TA)7/(TA)7]

study patients was 6–9 months. One patient had an acute relapse that was successfully treated with rectal corticosteroids, and another patient still has mildly active disease. The other four patients have remained in remission, in two of these clinical remission was confirmed endoscopically.

Table 4 Results of the laboratory examinations at the different dose levels in the non-study patients

Laboratory parameter	Azathioprine 50 mg/day Week 1–2	Azathioprine 50 mg/day + allopurinol 50 mg/day			Maintenance dose azathioprine 50 mg/day + allopurinol 50 mg/day Follow-up week 3–4
		Week 1	Week 2	Week 4	
6-TGN (pmol/8×10^8EC)	126 (63–177)	359 (175–482)	389 (243–629)	311 (274–401)	361 (304–458)
MMPN (pmol/8×10^8 EC)	497 (228–1,581)	223 (50–958)	342 (50–743)	380 (100–750)	441 (50–1,790)
Leukocytes (/μl)	5,640 (2,720–11,620)			5,900 (3,480–10,570)	6,000 (4,820–8,110)
Lymphocytes (/μl)	1,210 (360–1,990)			1,290 (340–2,350)	1,110 (520–2,350)
Bilirubin total (μmol/l)	7 (3–34)			6 (2–30)	7.5[a] (2–15)
ALT (U/l)	13 (12–25)			16 (13–29)	17 (12–25)
AST (U/l)	24 (19–30)			22 (19–40)	28 (20–35)

Normal ranges and abbreviations: See footnote to Table 1

Data are presented as the median ($n = 5$) with the range in parenthesis

[a] Value for one patient missing

Discussion

Our study provides evidence that lower doses than previously reported of both azathioprine and allopurinol are sufficient to achieve therapeutic 6-TGN concentrations and to reverse elevated MMPN concentrations and elevated liver enzymes in IBD patients with insufficient response to azathioprine monotherapy. In our patients, a maximum dose of 50 mg azathioprine in combination with 50 mg allopurinol was sufficient to achieve biochemical response. This low-dose combination was adequate not only in the setting of a clinical study, but also in patients who did not meet the strict study inclusion criteria.

The use of thiopurines is limited due to a lack of response or side effects in approximately one-third of IBD patients [23, 24], and 10–15 % of patients do not achieve therapeutic 6-TGN concentrations and have preferential MMPN metabolism [1–4]. Several studies have reported the favorable metabolic interaction between thiopurines and allopurinol in IBD patients—if thiopurine doses are reduced to avoid excessive bone marrow toxicity. Dosing in these studies was usually based on recommendations from the literature. Typically, thiopurine doses were reduced to 25–50 % of the initial thiopurine dose and combined with allopurinol 100–300 mg qd [6–9, 12–15]. In one retrospective chart analysis, a starting dose of allopurinol 50 mg qd in combination with thiopurines was recommended [25]. Not only hepatotoxicity but also myelosuppression is one of the most relevant side effects of thiopurine therapy. In several of the thiopurine–allopurinol combination trials, leukopenia rates ranged between 20 and 50 % [6, 7, 12, 14]. None of our patients experienced leukopenia or neutropenia and, overall, the combination was well tolerated. In one patient with an acute UC flare after the azathioprine dose had been reduced to 50 mg qd, transaminases increased after the initiation of oral prednisone treatment; this increase was probably not related to allopurinol but to prednisone. Considering the overall good tolerability in our patients, lower dosing might be a helpful means to reduce toxicity rates.

There are, however, a number of unresolved questions about azathioprine dose adaptations. In our study, we defined a target 6-TGN concentration limit of 350 pmol/8×10^8EC; this had to be attained to begin with the follow-up period and to stop dose-escalation. According to the literature, the therapeutic range of 6-TGN concentrations is 235–450 pmol/8×10^8EC [1, 21, 26]. However, the upper limit of 450 pmol/8×10^8EC is not well corroborated by experimental data. As thiopurine concentrations show a high variability between two measurements, the target of 350 pmol/8×10^8EC was chosen to ensure that the concentrations were reliably within the therapeutic range when the decision on further dose increase or initiation of the follow-up was made. Additionally, this value is roughly the mean of the therapeutic range.

The mechanism of how allopurinol increases 6-TGN concentrations and at the same time lowers MMPN concentrations remains to be elucidated. An effect of allopurinol on TPMT activity could neither be demonstrated in vitro nor in vivo [6, 7], which is in accordance with our observation that TPMT activity in the study patients did not change. Furthermore, current data do not support the hypothesis that allopurinol metabolites inhibit TPMT activity and thus decrease MMPN production [7]. Other, so far unproven explanations might be the induction of other enzymes, such as hypoxanthine guanine phosphoribosyltransferase and/or of enzymes which biotransform MMPNs, or the inhibition of unknown TPMT co-enzymes by allopurinol or its metabolites. Alternatively, the mere reduction of azathioprine dose may interfere with the activation of TPMT and thus lower MMPN concentrations simply by decreasing the azathioprine burden. To our knowledge, we are the first to perform an

azathioprine dose-reduction phase prior to combination with allopurinol. MMPN concentrations were already normalized in all of our patients after the azathioprine dose had been reduced to 50 mg qd. Since the median MMPN remained stable after the addition of allopurinol to the therapeutic regimen, this observation supports the hypothesis that reduction of the azathioprine dose burden is responsible for the rapid normalization of MMPN concentrations.

Three of five patients who completed the study needed oral and/or rectal corticosteroids to be in remission at inclusion, and one patient had active disease despite oral prednisone intake. All patients had one or more episodes of corticosteroid intake in the months preceding study entry. Thus, despite formally normal disease activity indices in most patients at inclusion, response to thiopurine monotherapy was unsatisfactory. Likewise, all non-study patients had clinically active disease prior to the addition of allopurinol to the therapeutic regimen. In conclusion, the good response to the allopurinol–azathioprine combination can not be explained simply by a mild disease activity prior to allopurinol addition.

Up to the present, seven of the ten patients remain in remission based on clinical and/or endoscopic findings. This number is in accordance with data from the literature describing clinical response rates for the thiopurine–allopurinol combination of >70 % [7, 9, 12]. However, our patients took lower doses to reach the same result. Follow-up periods of up to 71 months [8] have shown that thiopurines and allopurinol can be safely and effectively combined for long time periods.

After study completion metabolite concentrations were controlled every 3–6 months in our patients. Without any obvious explanation, in one CU patient, 6-TGN concentrations continued to rise above 500 pmol/8×10^8EC after approximately 6 months of combination therapy. This led to the azathioprine being reduced to an alternating dose of 50 mg and 25 mg qd, while allopurinol was maintained at 50 mg qd. According to the literature, the estimated half-life of 6-TGN in EC is 3–13 days, with an average of about 5 days [17, 19, 27]. However, much longer half-lives have been reported [28], and our observation in one patient may be explained by 6-TGN accumulation caused by prolonged elimination.

We decided to apply the allopurinol–azathioprine combination therapy in five patients who did not fulfil the study inclusion criteria, as all five patients were insufficiently treated with azathioprine alone. The aim was to avoid a therapy escalation to a TNF-alpha blocker. With the exception of xanthine oxidase inhibitors, such as allopurinol, and aminosalicylates, which inhibit TPMT activity in high doses [29], no pharmacokinetic drug interactions affecting thiopurine metabolite concentrations are known for azathioprine. Thus, the concomitant medications should not have had any significant influence on azathioprine pharmacokinetics.

Seven patients had lymphopenia at follow-up, which is a recognized effect of thiopurines [30]. One of these patients received corticosteroids at follow-up, which may have contributed to the lymphopenia. In the patient with the lowest lymphocyte count at inclusion (750/μl), lymphocytes dropped to as low as 320/μl, but finally reached stable values of approximately 500/μl at follow-up. Lymphopenia-related complications, such as infections, did not occur in any of the patients. Interestingly, two of the patients with normal lymphocyte counts relapsed.

A shortcoming of our study is the low number of patients. However, in view of the consistency of the clinical and biochemical results within and outside the study protocol, the number of relapses, which is comparable to treatments with higher doses, and the length of follow-up, it may be concluded that low doses of 50 mg allopurinol in combination with 50 mg azathioprine qd are sufficient in most IBD patients with inadequate thiopurine metabolite concentrations. Even in patients with relevant comorbidities, the low-dose combination proved to be efficacious and safe. Tolerability can be optimized and thiopurine-induced toxicity, such as leukopenia, can be prevented by lowering doses to the required minimum. Further studies are necessary to confirm our findings in larger patient groups and to evaluate if the low-dose combination may also prevent toxicity and improve efficacy in thiopurine-naïve patients.

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