Eur J Med Res (2006) 10: 535-538

The Leukocyte Count Predicts the Efficacy of Treatment with Azathioprine in Inflammatory Bowel Disease*

J. Glas[1,3] , H.-P. Török[1,2], J. Daczo[1,2], L.Tonenchi[1,3], M. Folwaczny[3], C. Folwaczny[1,2]

[1]Medizinische Poliklinik - Innenstadt, [2]Chirurgische Klinik und Poliklinik - Innenstadt,
[3]Poliklinik für Zahnerhaltung und Parodontologie, Klinikum der Universität München, Munich, Germany

Abstract
Introduction: Azathioprine has variable efficacy in inflammatory bowel disease. Previous studies suggested that either neutropenia, an increase in the mean corpuscular volume, the assessment of thiopurine methyltransferase activity or erythrocyte 6-thioguanine values might predict the treatment response. However, due to the conflicting results of the preceding studies there are yet no established laboratory values which allow an estimation of the clinical response.
Patients and methods: 45 patients with Crohn's disease and 39 patients with ulcerative colitis were enrolled in this retrospective evaluation. After a minimum of six months therapy with azathioprine patients in remission were compared with those who did not achieve a stable remission with respect to the number of leucocytes, lymphocytes, neutrophil granulocytes and the mean corpuscular volume.
Results: Patients who went into remission during treatment with azathioprine displayed significantly lower leukocyte counts if compared to patients who were not in remission ($p = 0.004$ in Crohn's disease and 0.003 in ulcerative colitis). A similar tendency was also observed with respect to the granulocyte count ($p = 0.007$ in Crohn's disease and 0.004 in ulcerative colitis). The mean corpuscular volume did not correlate with the response to purine analogues.
Discussion: The absolute leukocyte count and the percentage of granulocytes seem to predict the response to purine analogues in inflammatory bowel disease and possibly offers a feasible and cost effective diagnostic tool for the assessment of therapeutic efficacy. Subsequent preferably prospective studies should aim to define the optimal cut-off value for the leukocyte count.

Introduction

Azathioprine and 6-mercaptopurine are widely used for the treatment of both Crohn's disease and ulcerative colitis. Clinical trial data and a meta-analysis have confirmed the efficacy of azathioprine for Crohn's disease [1-4]. There are less efficacy data for ulcerative colitis and there are few data that have compared remission and relapse rates for ulcerative colitis and Crohn's disease [5-8].

Purine analogues undergo extensive enzymatic and non-enzymatic metabolism. Azathioprine is a prodrug, which is non-enzymatically converted into 6-mercaptopurine, a compound, that can then be metabolized to one of three metabolites by competing pathways [9]. 6-methylmercaptopurine is produced by thiopurine methyl-transferase (TPMT) and is thought to be inactive, although some authors have suggested an active role in drug toxicity [9]. 6-thiouric acid is an inactive metabolite, which is formed by xanthine oxidase and is excreted renally. Finally, 6-thioguanine nucleotides (6-TGNs) are formed by hypoxanthine-guanine phosphoribosyl-transferase (HGPRT) and are subsequently incorporated into DNA and RNA. 6-TGNs are the putative active metabolites and are also associated with some dose related adverse effects [9]. The relative activities of HGPRT, xanthine oxidase, and TPMT determine the net concentration of 6-TGNs produced. Within the xanthine oxidase gene no polymorphisms are known so far, but up to 2% of the general population have xanthine oxidase deficiency, giving rise to hypouricaemia. Although no polymorphisms in the HGPRT gene have been documented, the Lesch-Nyhan syndrome is characterized by a constitutive lack of activity of HGPRT. In contrast, the pharmacogenetic influence of TPMT polymorphisms is well established. In TPMT deficient patients, increased production of 6-TGNs leads to toxic levels in blood cells, causing pancytopenia or isolated leukopenia [9]. However, allelic variants of TPMT do not explain all haematotoxic events [10].

The assessment of TPMT activities or 6-TGN concentrations were repeatedly suggested for the prediction of therapeutic efficacy of purine analogues in inflammatory bowel disease [10]. However, preceding trials yielded conflicting results and the measurement of TPMT activities or 6-TGN concentrations is costly and time consuming. Due to a possible impact of azathioprine, 6-mercaptopurine or mesalamine, on TPMT activity, it is yet unclear, when these parameters should be assessed ideally, e. g. before or during the treatment with purine analogues [10]. Thus far, few trials investigated the role of the white blood cell count [11-13] or an increase in the mean corpuscular volume [14], which would be apparently feasible tools for defining the optimal dose of azathioprine or 6-mercaptopurine. Thus, the present study compared the white blood cell count and the mean corpuscular volume with the induction of remission in patients with Crohn's disease

*This work contains parts of the doctoral thesis of Jürgen Daczo.

or ulcerative colitis, who had received azathioprine for at least 6 months.

Patients and Methods

The charts of all patients with Crohn's disease or ulcerative colitis attending the outpatient department at the university hospital of Munich - Standort Innenstadt, between 1991 and 2002 were reviewed. Patients were eligible for this retrospective evaluation if azathioprine therapy had been initiated and continued for at least six months. Patients who had started azathioprine treatment at another hospital were not enrolled. Patients with an indeterminate colitis or patients, who received azathioprine primarily for other indications (renal transplant, rheumatoid arthritis, autoimmune liver disease), were excluded. Patients, who had received ciclosporine or infliximab, were also not eligible. Remission was defined as no need for oral steroids (either prednisolone or budenoside) for at least three months and a Harvey-Bradshaw score of 4 or less. Patients who were well on low doses of steroids were reported as "remission not achieved". The continued use of oral 5-aminosalicylic acid compounds and steroid or 5-aminosalicylic acid enemas was allowed within the definition of remission. Relapse was defined as the need for reintroduction of steroids or the need for a surgical procedure. In Crohn's disease disease extent and behavior was classified according to the Vienna classification [15] (for details see Table 1). For comparison of the leukocyte count and MCV values between patients with and without remission under azathioprine the lowest (leukocyte count) or the highest (mean corpuscular volume) documented value were employed. In addition, the minimal percentage of lymphocytes and granulocytes during the evaluation period were also recorded. Statistical analysis was performed by applying the Mann-Whitney-U or the unpaired t-test for parametric data. Non-parametric data were compared employing the Fisher's exact test.

Results

45 patients with Crohn's disease were enrolled and 14 (31%) achieved a remission under therapy with azathioprine. 39 patients with ulcerative colitis had been treated with azathioprine of whom 13 went into remission (33%). Depending on the presence or absence of remission during treatment with azathioprine the baseline characteristics of patients with Crohn's disease or ulcerative colitis are depicted in Tables 1 and 2, respectively.

In Crohn's disease patients, who went into remission during treatment with purine analogues, displayed significantly lower leukocyte counts if compared to patients who were not in remission (5.3 ± 2.1 vs. $8.9 \pm 3.9 \times 10^3/\mu l$; $p = 0.004$). A similar tendency was also observed in ulcerative colitis (6.0 ± 1.1 vs. $8.8 \pm 2.4 \pm 10^3/ml$; $p = 0.003$). In Crohn's disease and ulcerative colitis the percentage of neutrophils was also significantly lower in patients who achieved remission, whereas the percentage of lymphocytes was significantly increased. The mean corpuscular volume did not correlate with the response to azathioprine (Table 3).

Discussion

The present study confirms the efficacy of azathioprine for therapy of both Crohn's disease and ulcerative colitis. The remission rates achieved herein and the reported acceptable maintenance of remission with ongoing treatment make azathioprine a valuable part of the treatment of inflammatory bowel disease. These results are consistent with clinical trial data [1-8]. A meta-analysis of randomized studies of azathio-

Table 1. Baseline characteristics of patients with Crohn's disease with (+) or without (-) remission after 6 months of therapy with azathioprine.

	Remission		p-value
	+	-	
Gender			
Male	4	10	0.805
Female	10	21	
Age			
Mean	42	40	0.544
Disease site			
terminal Ileum (L1)	5	9	
Colon (L2)	3	10	0.427
Ileocolonic (L3)	4	7	0.973
Stomach, Duodenum, Jejunum (L4)	1	2	0.937
Disease type			
Non-fistulizing, non-fibrostenotic (B1)	3	7	0.874
Fibrostenotic (B2)	5	4	0.135
Fistulizing (B3)	6	16	
Extraintestinal manifestations*			
Improvement	2	5	1.000
No Improvement	2	5	

*Arthralgias and cutaneous, ocular or hepatic involvement

Table 2. Baseline characteristics of patients with ulcerative colitis with (+) or without (-) remission after 6 months of therapy with azathioprine.

	Remission		p-value
	+	-	
Gender			
Male	8	15	0.719
Female	5	12	
Age			
Mean range	43	44	0.769
Disease site			
Distal	3	1	0.114
Left hemicolon	2	6	0.659
Pancolitis	8	16	
E**xtraintestinal manifestations***			
Improvement	4	7	0.922
No Improvement	1	2	

*Arthralgias and cutaneous, ocular or hepatic involvement

Table 3. Hematologic parameters (mean ± one Standard deviation (SD)) in Crohn's disease patients with (+) or without (-) remission during azathioprine therapy.

	Remission ± SD		p-value
	+	-	
Leukocyte count/µl	5350 ± 2106	8918 ± 3858	0.004
Lymphocytes (%)	16.6 ± 6.8	9.6 ± 5.8	0.005
Granulocytes (%)	73 ± 8.7	81 ± 6.9	0.007
MCV (fl)	93 ± 6.3	87 ± 15.1	0.221

Table 4. Hematologic parameters (mean ± one Standard deviation (SD)) in ulcerative colitis patients with (+) or without (-) remission during azathioprine therapy.

	Remission ± SD		p-value
	+	-	
Leukocyte count/µl	6045 ± 1165	8767 ± 2356	0.003
Lymphocytes (%)	20 ± 5.3	12 ± 5.6	0.001
Granulocytes (%)	69 ± 6.1	79 ± 8.9	0.004
MCV (fl)	92 ± 7.6	94 ± 8.5	0.543

prine treatment in Crohn's disease gave an odds ratio of 3.1 for inducing remission and an odds ratio of 2.3 for maintaining remission [4]. However, the comparably low remission rates observed herein are possibly due to the strict criteria, which were chosen for defining remission, that is 3 months without steroid therapy. A second reason may be the fact, that mean azathioprine dose in the patients evaluated herein was 1.6 mg/kg of body weight, which is at the low end of the recommended dosage [16]. Herein, azathioprine was also more likely to achieve remission in patients with ulcerative colitis than Crohn's disease, which is in accordance with literature data [14].

The white blood count was a good predictor of achieving remission, while the mean corpuscular volume was not. In a previous trial white blood count and mean cell volume were closely correlated but in an logistic regression analysis were identified as independent variables for predicting remission [14].

The present study was retrospective, which limits the validity of the evaluated data, although potential predictors of the treatment response such as age or disease behavior [14] were equally distributed between groups (Tables 1 and 2) and thus an observational bias is unlikely. Furthermore, the achieved data concerning the neutrophil count have modest clinical use because of the variable onset of fall in white blood count and significant overlap between responders and non-responders. In the first few months of azathioprine treatment there may be no change in white blood

count possibly because of the inflammatory activity and also because of steroid treatment. Moreover, some patients with a normal or "high normal" white blood count display a good response to azathioprine. It is debatable whether dose increases should be based on achieving a fall in white count, but its presence seems to be an encouraging sign for both the patient and the physician. In the study of Candy and co-workers leukopenia requiring dose reduction was associated with sustained remission [3]. Colonna and Korelitz also found a strong positive correlation between the extent of drug induced leukopenia and clinical outcome [11]. A low white blood count was also a significant variable for prediction of remaining in remission. However, in a recent retrospective trial Campbell and Ghosh addressed the question of whether neutropenia is required during therapy with azathioprine in patients with inflammatory bowel disease to reduce the relapse rate [13]. In this retrospective study of 173 patients with Crohn's disease or ulcerative colitis, respectively, on azathioprine, no difference in relapse rates was noted between patients with or without neutropenia. Of concern was the fact, that four of the 44 neutropenic patients stopped azathioprine because of severe life-threatening neutropenia. However, it has been recognized, that neutrophils are important in the inflammatory response in patients with IBD during a clinical relapse. Mucosal biopsies reveal increased numbers of neutrophils in the lamina propria [17], and nuclear medicine studies showed increased activity in the bowel during clinical relapse [18]. Finally, the leukopenia, that is seen in patients taking azathioprine or 6-mercaptopurine, is characterized by the preferential suppression of neutrophils [19]. Although the importance of leukopenia remains somewhat unclear in relation to the optimization of therapy with purine analogues, neutropenia is not a goal of therapy because of its potential harm, that can result from severe infections, and the white blood cell count has to be followed closely during early therapy to ensure against possible neutropenia.

In conclusion, even if one adheres to a strict definition of remission as chosen herein azathioprine is effective in the treatment of inflammatory bowel disease. A decrease in the leukocyte count associated with the use of purine analogues may be used as an indicator of therapeutic response to 6-mercaptopurine or azathioprine, but the induction of neutropenia has never been, and should not be, advocated as a goal of therapy.

REFERENCES

1. Present DH, Korelitz BI, Wisch N, et al. Treatment of Crohn's disease with 6-mercaptopurine. A long-term randomised double-blind study. N Eng J Med 1980;402: 981-987.
2. O'Donoghue DP, Dawson AM, Powell-Tuck K, et al. Double-blind withdrawal trial of azathioprine as maintenance treatment for Crohn's disease. Lancet 1978;2:955-957.
3. Candy S, Wright J, Gerber M, et al. A controlled double blind study of azathioprine in the management of Crohn's disease. Gut 1995; 37: 674-678.
4. Pearson DC, May GR, Fick GH, et al. Azathioprine and 6-mercaptopurine in Crohn's disease. A meta-analysis. Ann Intern Med 1995;123:132-142.
5. Kirk AP, Lennard-Jones JE. Controlled trial of azathioprine in chronic ulcerative colitis. BMJ 1982;284:1291-1292.
6. Rosenberg JL, Wall AJ, Levin B, et al. A controlled trial of azathioprine in the management of chronic ulcerative colitis. Gastroenterology 1975;69:96-69.
7. Jewell DP, Truelove SC. Azathioprine in ulcerative colitis: final report of a controlled therapeutic trial. BMJ 1974;4:627-630.
8. Hawthorne AB, Logan RFA, Hawkey CJ, et al. Randomised controlled trial of azathioprine withdrawal in ulcerative colitis. BMJ 1992;305:20-22.
9. Dubinsky MC. Azathioprine, 6-mercaptopurine in inflammatory bowel disease: pharmacology, efficacy, and safety. Clin Gastrooenterol Hepatol 2004;2:731-743.
10. Lichtenstein GR. Use of laboratory testing to guide 6-mercaptopurine/azathioprine therapy. Gastroenterology 2004;127:1558-1564.
11. Colonna T, Korelitz BI. The role of leukopenia in the 6-mercaptopurine-induced remission of refractory Crohn's disease. Am J Gastroenterol 1994;89:362-366.
12. Berg PS, George J, Present DH. 6-MP-is leukopenia required to induce to induce remission in the treatment of ulcerative colitis ? Gastroenterology 1996;110:A863.
13. Campbell S, Ghosh S. Is neutropenia required for effective maintenance of remission during azathioprine therapy in inflammatory bowel disease? Eur J Gastroenterol Hepatol 2001;13:1073-1076.
14. Fraser AG, Orchard TR, Jewell DP. The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. Gut 2002;50:485-489.
15. Gasche C, Scholmerich J, Brynskov J, et al. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna1998. Inflamm Bowel Dis 2000;6:8-15.
16. Hoffmann JC, Zeitz M, Bischoff SC, et al.. Diagnosis and therapy of ulcerative colitis: results of an evidence based consensus conference by the German society of Digestive and Metabolic Diseases and the competence network on inflammatory bowel disease. Z Gastroenterol 2004 42:979-983.
17. Teachon K, Bjarnason I. Comparison of leukocyte excretion and blood loss in inflammatory disease of the bowel. Gut 1993;34:1535-1538.
18. Burke DA, Dixon MF, Axon ATR. Ulcerative colitis; prolonged remission following azathioprine-induced pancytopenia. J Clin Gastroenterol 1989;11:327-330.
19. Korelitz BI, Zlatanic J, Smith MJ, et al. Significance of WBC differential when leukopenia is induced by 6-MP for IBD. Gastroenterology 1997;113:1810-1811.

Received: September 8, 2005 / Accepted: November 7, 2005

Address for correspondence:
PD Dr. med. Christian Folwaczny
Interdisziplinäre Molekulare Gastroenterologie
Medizinische Poliklinik - Innenstadt und
Chirurgische Klinik und Poliklinik - Innenstadt
Klinikum der Universität München
Nussbaumstrasse 20
D-80336 Munich, Germany
Tel.: +49-89/5160-2625
Fax: +49-89/5160-4187
E-mail: Christian.Folwaczny@med.uni-muenchen.de