Bone marrow toxicity caused by azathioprine in inflammatory bowel disease: 27 years of experience

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Abstract

Myelosuppression is an important and potentially lethal complication of azathioprine treatment. The blood count has been reviewed in all patients treated with azathioprine for inflammatory bowel disease over 27 years in one hospital. Altogether 739 patients (422 with Crohn's disease, 284 with ulcerative colitis, and 33 with indeterminate colitis) were treated with 2 mg/kg/day azathioprine for a median of 12·5 months (range 0·5–132) between 1964 and 1991. Full blood counts were performed monthly for the duration of treatment. In 37 patients (5%) who developed bone marrow toxicity, the drug was withdrawn or the dose reduced. Thirty two of these patients were asymptomatic and five developed symptoms. Leucopenia (white blood count less than $3·0 \times 10^9/l$) occurred in 28 (3·8%) patients, in nine of whom it was severe (white blood count $<2·0 \times 10^9/l$). Of these nine patients, three were pancytopenic: two died from sepsis and the other had pneumonia but recovered. A further two patients with severe leucopenia developed a mild upper respiratory infection only. Thrombocytopenia (platelet count $<100\ 000 \times 10^6/l$) in 15 patients was associated with leucopenia in six and developed in isolation in a further nine (total 2%). Isolated thrombocytopenia was never clinically severe. Myelotoxicity from azathioprine developed at any time during drug treatment (range 2 weeks–11 years after starting the drug) and occurred either suddenly or over several months. Bone marrow suppression as a result of azathioprine treatment is uncommon when a moderate dose is used, but is potentially severe. Leucopenia is the commonest and most important haematological complication. Regular monitoring of the full blood count is recommended during treatment.

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Azathioprine is well established as an effective drug in the treatment of inflammatory bowel disease. Although its usefulness was not shown in four controlled studies of patients with active Crohn's disease or ulcerative colitis,[1–4] a further six controlled trials examining this drug or its metabolite, 6-mercaptopurine, in chronic active Crohn's disease and ulcerative colitis have shown steroid sparing effects in all subjects and a clear therapeutic advantage in most.[5–10] Additional reports of uncontrolled studies support this impression.[11–16]

Concerns about the safety of azathioprine and 6-mercaptopurine, remain, however. Considerable clinical experience of azathioprine has now been obtained in organ transplantation, rheuma-

toid arthritis, and inflammatory bowel disease. The commonest sudden side effects include nausea, fever, pancreatitis, and bone marrow suppression.[17] One large study of 1349 patients with a variety of immunological diseases not associated with transplant showed a slightly increased risk of neoplasia, but the extent to which the drug alone was responsible was uncertain.[18]

The most common but potentially serious side effect is bone marrow suppression. The effect of azathioprine on bone marrow is largely dose-dependent. A conventional immunosuppressive dose of less than 2·5 mg/k/day causes predictable macrocytosis, a raised mean corpuscular haemoglobin concentration, and mild leucopenia.[19][20] Rarely, sudden, severe, and unexpected myelosuppression, which is possibly idiosyncratic, has also been reported when low to moderate doses (<2 mg/kg/day) of the drug have been used.[20–22] This myelosuppression consists of severe leucopenia, thrombocytopenia, and sometimes pancytopenia.

In a retrospective study of 396 patients with inflammatory bowel disease who were treated with 6-mercaptopurine, 2% developed clinically severe leucopenia (white blood cell count $<2·5 \times 10^9/l$).[17] Our experience with azathioprine in a similar group of patients extends over 27 years. All patients treated with this drug had their peripheral blood count monitored each month, providing a record of all abnormal counts and clinically adverse effects related to marrow suppression. We present the results of this experience in this report.

Methods

Between 1964 and 1991 760 patients at St Mark's hospital were treated with azathioprine at a standard dose of 2 mg/kg. Each subject was reviewed regularly as an outpatient during treatment and full blood counts were obtained monthly. Some patients who had been treated with azathioprine for several years had blood tests every two months.

A record was maintained for every patient treated with azathioprine, detailing any haematological or other side effects as well as other reasons for stopping or changing the dose of the drug.

The normal white blood count range at this hospital is $4–11 \times 10^9/l$. In this study, leucopenia is defined as moderate (white blood count $2·0–3·0 \times 10^9/l$) or severe ($<2·0 \times 10^9/l$). Thrombocytopenia refers to a platelet count less than $100\ 000 \times 10^6/l$.

For the purposes of this study, 21 patients were have been excluded from the analysis – five because of poor drug compliance or inadequate

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TABLE I   Clinical details of 739 patients treated with azathioprine

	Crohn's disease	Ulcerative colitis	Indeterminate colitis	Total
No of patients	422	284	33	739
Male	204	152	14	370
Female	218	132	19	369
Median duration of treatment (mth)	14·0	9·0	16·0	12·0
Range (mth)	0·01–183	0·1–171	0·5–99	0·01–183
Total no of patients – months of treatment	12 867	5757	839	19 463

TABLE II   Patients with Crohn's disease (CD) and ulcerative colitis (UC) who developed bone marrow suppression while taking azathioprine

	Leucopenia		Leucopenia* and thrombocytopenia†		Thrombocytopenia	
	CD	UC	CD	UC	CD	UC
No of patients	16	6	4	2	6	3
Male	5	2	4	2	3	3
Female	11	4	–	–	3	–
Mean age at onset of myelotoxicity (y)	36	33	34	18	45	38

Leucopenia: white blood count <3·0×10⁹/l; thrombocytopenia – platelet count <100 000×10⁶/l.

The number of months' treatment with azathioprine before the onset of severe (white blood count <2·0×10⁹/l) or moderate (white blood count 2·0–3·0×10⁹/l) leucopenia and thrombocytopenia (platelet count <100 000×10⁶/l). Medians are indicated ——.

follow up, 10 in whom treatment was monitored at other centres, five who were treated with azathioprine for conditions other than inflammatory bowel disease, and one who was inadvertently given an excessive dose of the drug (6 mg/kg). Blood counts of the remaining 739 subjects who form the basis of this report were examined to identify all haematological reactions and case notes were reviewed for those with abnormalities.

Clinical indications for azathioprine treatment included long term steroid use, failure to control symptoms with convential treatment, and the presence of internal or external fistulae. When required, patients continued to receive other conventional anti-inflammatory agents including corticosteroids, sulphasalazine (or 5-acetyl-salicylic acid derivatives), or metronidazole. No patient received other immunosuppressive drugs.

Results

Altogether 422 patients had Crohn's disease, 284 ulcerative colitis, and 33 indeterminate colitis. The median duration of treatment with azathioprine was 12·0 (range 0·01–184) months. The total of patient years of treatment was 1622. The clinical details of these patients are given in Table I. One hundred and forty patients received the drug for only one month or less because of acute side effects or elective cessation of the drug in the early years of its use; none of these patients stopped taking the drug because of myelosuppression. Patients who developed myelosuppression had no clinical evidence of renal, hepatic, or splenic dysfunction beforehand.

Bone marrow toxicity, a white blood count <3·0×10⁹/l, a platelet count <100 000×10⁶/l, or pancytopenia caused by azathioprine occurred in 37 patients (5·0%). Their details are given in Table II. The drug was stopped in all except two patients in whom it was continued at a reduced dose. Leucopenia occurred in 28 patients (3·8%). In nine this was severe (white blood count <2·0×10⁹/l) and in 19 it was moderate (white blood count 2·0–3·0×10⁹/l). The neutrophil
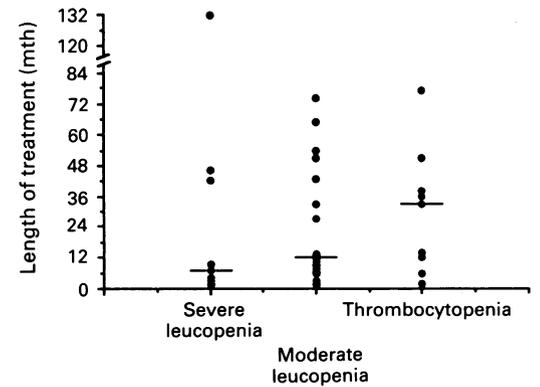
count was lower than the counts for other white cell lines in all of these patients. Three of them had severe leucopenia as part of pancytopenia. Another patient with severe leucopenia and two with moderate leucopenia also had associated mild thrombocytopenia (platelet count <100 000×10⁶/l). Isolated mild and asymptomatic thrombocytopenia (60 000–100 000× 10⁶/l) occurred in a further nine patients. A total of 15 patients (2·0%) experienced some degree of thrombocytopenia.

SYMPTOMS

Five patients (0·7%), all with severe leucopenia, had symptoms from myelotoxicity. Profound pancytopenia occurred in three, resulting in two deaths from sepsis and one serious lung infection which resolved eventually. Two others with severe leuopenia developed mild upper respiratory infections which responded to oral antibiotics and withdrawal of azathioprine. The clinical details of one of the patients who died have been described previously.[9]

TIMING OF BONE MARROW TOXICITY IN RELATION TO LENGTH OF TREATMENT

Although bone marrow suppression occurred at any time during treatment, it generally developed early (Fig 1). The nine patients with severe leucopenia, including the three with pancytopenia, developed myelotoxicity after 0·5–132 months of treatment (median 9 months). This complication occurred within three months in four patients and within a year in a further two. Myelotoxicity in the 19 patients with moderate leucopenia developed after 1–76 (median 12·5) months of treatment. Isolated thrombocytopenia developed after 2–51 months of drug treatment.

RAPIDITY OF ONSET OF BONE MARROW SUPPRESSION

Severe leucopenia occurred abruptly in six of the nine patients; blood counts one month previously had been normal in these cases. The other three patients developed severe leucopenia gradually over more than two months; initial reductions in the white blood count were overlooked and the

drug dose was changed only when severe leucopenia occurred.

Four of the five symptomatic patients, including the three with pancytopenia, developed severe leucopenia suddenly; their blood counts one month earlier had been normal. A gradual and progressive fall in the white blood count occurred in the fifth, symptomatic patient who presented with a mild respiratory infection associated with severe leucopenia.

All but three of the 19 patients who developed moderate leucopenia had had normal white blood cell counts one month earlier. Two of these 16 patients had had a slight fall in their platelet count, to just below 100 000×10⁶/l, one month before their white blood cell count became abnormal. Myelosuppression was progressive in the three other patients in whom azathioprine was continued despite the presence of moderate leucopenia.

Isolated thrombocytopenia occurred suddenly in six of nine patients. Their platelet and other blood counts had been well within the normal range previously. In the other three patients there was a gradual decline in the platelet count over two or more months.

RECOVERY FROM MYELOTOXICITY
Apart from the two patients who died, the full blood counts returned to normal within one month in all patients who had myelotoxicity as a result of azathioprine treatment, including the patient with pancytopenia and severe pneumonia.

SUBSEQUENT TREATMENT WITH AZATHIOPRINE
Azathioprine was reintroduced to 19 patients who had developed myelotoxicity. Two of the nine patients with severe leucopenia resumed the drug at a lower dose; one had an uncomplicated course over several years, the other enjoyed good health for a further 10 years until the drug was stopped electively. Nine years later, however, this 74 year old woman has developed possible early myelodysplastic syndrome.

Thirteen of 19 patients with moderate leucopenia resumed azathioprine. Three of these were treated again at the same dose and all developed moderate leucopenia again. The other 10 patients were restarted at a lower dose and two of them developed recurrent mild leucopenia. The drug was stopped in those with recurrent leucopenia.

A further patient with combined mild leucopenia and thrombocytopenia developed these side effects again when azathioprine was reintroduced at an identical dose. Three patients with isolated thrombocytopenia were restarted on azathioprine at a reduced dose: none of these developed any subsequent abnormality of the full blood count.

Discussion
Azathioprine is a synthetic analogue of the naturally occurring purine, adenosine. It is converted to 6-mercaptopurine and since their subsequent metabolism is identical, both drugs share similar clinical and side effects. The dose of 6-mercaptopurine required to produce equivalent therapeutic and toxic effects as azathioprine is about 55% that of azathioprine.[23] These drugs possess both cytotoxic and immunosuppressive properties. Cytotoxicity is believed to result from the incorporation of thiopurine metabolites into cellular nucleic acids, while the immunosuppressive effects are secondary to inhibition of de novo purine ribonucleotide synthesis and interconversion.[24]

Mild leucopenia, defined as a white cell count between 3·0 and 4·0×10⁹/l, is the commonest haematological side effect of azathioprine. This effect is thought to be dose-dependent and relates to a shift towards less mature forms and a decreased myeloid-erythroid ratio.[19] The principal effect of this drug on white blood cells is granulocytopenia, which occurs shortly after starting azathioprine. Accompanying bone marrow changes on granulopoiesis are not seen until the peripheral blood count falls below 2·0×10⁹/l.[25] More profound bone marrow suppression occurs with higher doses of azathioprine or can develop unexpectedly as an idiosyncratic reaction. In these cases, agranulocytosis or pancytopenia results from disturbed marrow cellularity and impaired cell line maturation.[25]

The risk of leucopenia in inflammatory bowel disease as a result of azathioprine or 6-mercaptopurine treatment is reported to be 2 to 5%, though few studies define the term leucopenia clearly.[12 13 15-17 26] In the National Crohn's Disease Cooperative Study, 15% of patients treated with 2·5 mg/kg developed a white blood cell count less than 4·0×10⁹/l. In the same study, treatment with 1·0 mg/kg azathioprine was associated with a 2% incidence of leucopenia.[27] In another study, 2% of 396 patients treated with 6-mercaptopurine in a dose of 1·5 mg/kg/day developed a white blood cell count of less than 2·5×10⁹.[17] In a meta-analysis of 542 patients treated with azathioprine for rheumatoid arthritis, 14 patients (2·6%) developed a white blood count <2·5×10⁹/l and a further 34 patients (6·3%) developed a count of between 2·5 and 3·5×10⁹/l.[26]

Other effects of azathioprine are seen in the peripheral blood. Macrocytosis is common; it occurs in approximately 70% of renal transplant recipients.[28 29] Reversible erythroid hypoplasia has been reported in one patient given 6-mercaptopurine for Crohn's disease and four renal transplant recipients treated with azathioprine.[17 30] In keeping with our own findings, previous studies have shown that thrombocytopenia occurs less commonly than leucopenia and is not usually of clinical severity.[17 26 31]

To our knowledge five detailed case reports of pancytopenia related to the use of azathioprine for immunosuppression have been published. Four patients with connective tissue disease and one with ulcerative colitis developed reversible pancytopenia after 0·5-15 months of therapy with 75-100 mg/day azathioprine.[20 21 32] Further reports of pancytopenia have been reported to the drug's manufacturers.[19] There has been only one reported death related to bone marow toxicity from immunosuppressive azathioprine treatment.[33]

The reasons for individual variation in bone marrow susceptibility to azathioprine deserve attention. Bone marrow toxicity seems to be related to the excessive intracellular concentration of the cytotoxic active metabolities 6-thioguanine nucleotides.[22] These substances may accumulate if the activity of the catalytic enzyme thiopurine methyltransferase is deficient.

An inverse relationship between the red cell concentration of 6-thioguanine nucleotides and the neutrophil count has been reported in children given azathioprine for leukemia.[34] Furthermore, five patients who developed acute myelosuppression after taking azathioprine at a dose of 1·0–2·5 mg/day showed very low thiopurine methyltransferase activities and abnormally high 6-thioguanine nucleotide concentrations compared with 16 patients who were also receiving azathioprine but did not have myelosuppression.[22]

Further cases of patients with reduced thiopurine methyltransferase activity and leucopenia secondary to azathioprine or 6-mercaptopurine provide supporting evidence that low thiopurine methyltransferase activity may be a major risk factor for azathioprine induced myelosuppression.[35-36]

The activity of thiopurine methyltransferase seems to be controlled by a common genetic polymorphism. One in 300 individuals in the population has very low or no enzyme activity and 11% have intermediate activity.[37] Homozygotes and heterozygotes for this enzyme may have different thiopurine methyltransferase activity and therefore different susceptibility to azathioprine toxicity. The information on thiopurine methyltransferase and susceptibility to myelotoxicity from azathioprine is relatively recent, and post dates most of the data collected in this series. Testing for thiopurine methyltransferase activity is not routinely available at present, though it is possible to measure serum 6-thioguanine nucleotide concentrations.

Our experience with azathioprine in inflammatory bowel disease confirms that the risk of myelotoxicity is low but that this has potentially serious clinical sequelae. Leucopenia is the commonest and most serious toxic effect of azathioprine on the bone marrow. All five patients who developed symptoms related to bone marrow suppression were severely leucopenic. None of the patients with moderate leucopenia had symptoms. In other words, warning signs in the form of symptoms did not occur until myelosuppression was advanced.

Of the 37 patients with myelosuppression, 28 developed this complication within one month of a normal full blood count and the drug was stopped or its dose reduced. Twenty four of these patients were asymptomatic and it is not known how many would have gone on to develop more severe myelosuppression had the drug been continued at the same dose. Myelotoxicity was not recognised initially in the other nine patients and azathioprine was continued at the same dose; a progressive deterioration in the blood count occurred over the next month until the drug was stopped.

The deaths of two patients, after three and 132 months of azathioprine treatment, show that serious bone marrow toxicity is unpredictable. Complications affecting the bone marrow had a very variable time of onset after starting treatment in the other 35 patients with myelosuppression. Whilst reduced thiopurine methyltransferase activities may be responsible for azathioprine-induced myelotoxicity in some patients, the late onset of this complication in others suggests that additional factors must also be involved.

Both leucopenia and thrombocytopenia may recur with subsequent courses of azathioprine, particularly if the same dose is used; it is less likely to develop if dose reductions are made.

It seems prudent to stop the drug if moderate suppression of the white blood cell or platelet count occurs. We feel that azathioprine should never be used without blood tests; intervention only when symptoms occur may be too late. It could be argued that regular monitoring of the peripheral blood count did not influence the course of myelosuppression in our patients. In six of the nine patients with severe leucopenia the onset was sudden and unpredictable. This includes the two patients who died and the patient with pneumonia. However, the development of severe leucopenia could have been averted in the other three patients with more careful attention to the serial blood counts.

Routine blood testing throughout the duration of azathioprine treatment requires close and committed coordination between specialist, general practitioner, laboratory, and patient. An azathioprine card containing details of dose, possible side effects, and blood testing requirements should be given to patients taking this drug. The manufacturers of azathioprine recommend weekly blood testing for the first few months of treatment and three monthly or less thereafter. We believe that in inflammatory bowel disease where immunosuppressive doses of azathioprine are used, two weekly blood tests in the first three months of therapy are sufficient. Since the onset of myelosuppression is unpredictable, and often abrupt, we advocate more frequent testing of the blood count than three monthly for the remainder of treatment.

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