Utilisation of erythrocyte 6-thioguanine metabolite levels to optimise azathioprine therapy in patients with inflammatory bowel disease

C Cuffari, S Hunt, T Bayless

Abstract

***Background and aim*—The immunosuppressive properties of 6-mercaptopurine and its parent compound azathioprine are mediated by their intracellular metabolism into active 6-thioguanine (6-TG) metabolites. Measurement of erythrocyte 6-TG metabolite levels has been proposed as a useful clinical tool for assessing treatment efficacy in patients with inflammatory bowel disease (IBD).**

***Aim*—The purpose of the study was to establish a therapeutic index of treatment efficacy based on measurement of erythrocyte 6-TG metabolite levels, and apply it clinically to guide therapy.**

***Methods*—Heparinised blood was obtained from 82 adult patients with IBD on long term (more than three months) antimetabolite therapy (63 Crohn's disease; 19 ulcerative colitis). Erythrocyte 6-TG metabolite levels were measured using reverse phase high performance chromatography, and correlated with treatment efficacy. In 22 patients with refractory Crohn's disease despite long term azathioprine therapy, their dosage was increased by 25 mg/day at eight week intervals as needed. Serial erythrocyte 6-TG metabolite levels were measured at each clinic visit and correlated with treatment efficacy.**

***Results*—Clinical remission, as defined by a low disease index score in patients weaned off or on a low alternate day dose (<20 mg on alternate days) of corticosteroid, was achieved in 68% of patients on long term antimetabolite therapy. Treatment efficacy correlated with erythrocyte 6-TG levels greater than 250 pmol/8×10^8 red blood cells in patients with colonic and fistulising Crohn's disease ($p<0.01$) but not in patients with ileocolonic disease. Eighteen of 22 patients with incompletely responsive Crohn's disease achieved disease remission by optimising their dose of azathioprine therapy. Median (range) erythrocyte 6-TG metabolite levels increased from 194 (67–688) to 303 (67–737) pmol/8×10^8 red blood cells ($p<0.05$). Clinical response associated well with a reduction in corticosteroid requirements. Mean (SEM) white blood cell count decreased from 8.6 (0.9) to 6.9 (0.6) ×10^3/µl with adjustment in azathioprine dosage. No patient incurred azathioprine induced leucopenia.**

***Conclusion*—Measurement of erythrocyte 6-TG metabolite levels is helpful in determining the adequacy of azathioprine dosage and can be used to optimise the dose of antimetabolite therapy to achieve an improved clinical response without inducing leucopenia. Patients who are clinically refractory to azathioprine therapy despite achieving high erythrocyte 6-TG levels (>250) should be considered for adjunct or alternative forms of immunosuppressive therapy or surgery.**

(*Gut* 2001;**48**:642–646)

Keywords: azathioprine; metabolites; inflammatory bowel disease

Division of Gastroenterology, Department of Paediatrics, The Johns Hopkins Hospital, Baltimore, MD, USA
C Cuffari

Division of Gastroenterology, Department of Medicine, The Johns Hopkins Hospital, Baltimore, MD, USA
S Hunt
T Bayless

Correspondence to:
C Cuffari, The Johns Hopkins Hospital, Department of Pediatrics, Division of Gastroenterology, 600N, Wolfe St, Brady 308, Baltimore, MD 21287, USA.
ccuffari@jhmi.edu

Accepted for publication
5 December 2000

Azathioprine (AZA) 1.5 mg/kg/day and its metabolite 6-mercaptopurine (6-MP) 1 mg/kg/day have been proved to be efficacious in achieving and maintaining remission in 70% of patients with steroid dependant Crohn's disease.[1] Despite proven clinical efficacy, not all patients respond favourably, raising the possibility of either inadequate drug dosing or variations in disease severity.[2]

Currently, most physicians measure treatment efficacy by an improvement in clinical symptoms and quality of life or the patient's ability to remain in remission while weaning corticosteroid therapy.[3–5] Even with a dose of 2–2.5 mg/kg/day it may take as short as seven weeks or as long as 14 weeks before a clinically observable therapeutic effect occurs.[1] Although clinical response time may vary, at least 30% of patients with steroid refractory Crohn's disease fail to respond to the standard dosages used in most published studies.[1] The wide dose range of AZA used in clinical practice today would suggest that a safe and established therapeutic dose has not yet been defined.

As AZA and 6-MP are by themselves inactive, they must be transformed intracellularly into ribonucleotides that function as purine antagonists. These antimetabolites are then incorporated into DNA,[6] inducing cytotoxicity and immunosuppression.[7] 6-MP undergoes rapid and extensive catabolic oxidation to 6-thiouric acid in the intestinal mucosa and

Abbreviations used in this paper: IBD, inflammatory bowel disease; AZA, azathioprine; 6-MP, 6-mercaptopurine; 6-TG, 6-thioguanine; 6-MMP, 6-methyl mercaptopurine; TPMT, thiopurine methyl transferase; HPLC, high performance liquid chromatography; RBC, red blood cell; 5-ASA, 5-aminosalicylate; HBI, Harvey-Bradshaw index; WBC, white blood cell count.

liver by the enzyme xanthine oxidase. As proof of the complex metabolism, the bioavailability of 6-MP ranges from 5% to 37%.[7] In comparison, intestinal absorption of AZA is somewhat better than 6-MP. Once absorbed into the circulation, AZA is rapidly converted to 6-MP (88%) and S-methyl-4-nitro-5-thioimidazole (12%) on exposure to sulphydryl containing compounds in plasma and tissues. As AZA is 55% 6-MP by molecular weight, a conversion factor of 2.08 is used when comparing pharmacological dosages.[8]

Because of the rapid absorption of 6-MP into erythrocytes and organ tissues, the plasma half-life of 6-MP is very short, ranging from one to two hours.[9] The anabolic transformation of 6-MP into its active metabolites occurs intracellularly along the competing routes catalysed by thiopurine methyl transferase (TPMT) and hypoxanthine phosphoribosyl transferase, giving rise to 6-methyl mercaptopurine (6-MMP), 6-methyl-thioinosine 5'-monophosphate, and 6-thioguanine (6-TG) nucleotides, respectively. 6-TG is considered the active metabolite.[10] Incorporation of 6-TG into lymphocyte DNA affects cellular function and induces cytotoxicity and immunosuppression.[11] The direct lymphocytotoxic properties of 6-MP form the basis of its use in the treatment of lymphoma and leukaemia. In those patients, low erythrocyte 6-TG levels have been associated with a low 6-MP dose and an increased risk of disease relapse.[12]

An apparent genetic polymorphism has been observed in TPMT activity, the key enzyme involved in the metabolism of AZA and 6-MP. As a result of the heterogeneity in TPMT activity, patients with either negligible (0.3%) or low (11%) enzyme activity levels may have an increased risk of drug toxicity and leucopenia. In those patients, 6-MP metabolism is shunted towards excessive production of active 6-TG nucleotides.[13] In contrast, 10% of individuals have high TPMT activity and are considered rapid metabolisers, shunting 6-MP metabolism away from 6-TG and into production of 6-MMP. In the treatment of leukaemia, patients with high TPMT activity were shown to be at an increased risk of disease relapse despite a presumed therapeutic drug dosing regimen.[12]

A recent controlled trial in Crohn's disease compared oral (2 mg/kg/day) AZA therapy with and without initiating a short course of high dose intravenous AZA therapy. The study was confined to individuals with upper normal or high levels of TPMT enzyme activity so that high dose intravenous AZA (40 mg/kg) could be studied safely in one arm of the study.[14] Even at 2 mg/kg/day of oral AZA therapy, only 20% of these rapid metabolisers achieved clinical remission, a clinical response lower than that reported in consecutive patient publications.[3–5] Moreover, the adverse events in this study were higher than those previously reported in other adult series using moderate doses of AZA therapy.[14 15] It is therefore not possible to select one dosage of either 6-MP or AZA that will be rapidly effective in all patients while avoiding drug induced leucopenia.[15] In theory, awareness of an individual's TPMT enzyme activity level prior to starting therapy could be used clinically to optimise therapy and improve clinical response time.

Measurement of erythrocyte AZA metabolites has been proposed as a useful clinical tool for measuring clinical efficacy, documenting patient compliance to therapy, and explaining some drug toxicity. In our preliminary studies in paediatric patients with Crohn's disease, high performance liquid chromatography (HPLC) measurement of 6-MP metabolite levels showed an inverse correlation between erythrocyte 6-TG levels and disease activity. Although a wide range of erythrocyte 6-TG levels were associated with clinical responsiveness to therapy, patients with high levels (>250 pmol/8×10^8 red blood cells (RBCs)) in that series were uniformly asymptomatic.[16]

The purpose of our prospective study in adults was to establish a "therapeutic window" of drug efficacy based on measurement of AZA metabolite levels that can be used clinically to guide therapy. Lacking the prior knowledge of each patient's TPMT enzyme activity levels, we used a moderate dose of AZA (1–1.5 mg/kg/day) to minimise potential drug induced side effects, including leucopenia. Erythrocyte 6-TG levels were used to monitor increased dosing requirements for patients deemed unresponsive to conventional drug treatment dosages.

Material and methods

PATIENTS

Eighty two patients (40 males; 42 females) with inflammatory bowel disease (IBD) (63 Crohn's disease; 19 ulcerative colitis) were studied at the Meyerhoff IBD Center. All were adults with a mean (range) age of 42 (17–77) years. The diagnosis of IBD was established by standard clinical, radiological, and histological techniques.[17] Among the 63 patients with Crohn's disease, 29 had ileocolonic disease, 15 of whom had previously undergone a bowel resection, 26 had colonic disease, and four had a bowel resection. As a high proportion of patients with ileocolonic Crohn's disease had undergone bowel resection, patients were stratified to determine if a past history of surgical intervention would bias our results in favour of metabolite testing. Eight patients had fistulising Crohn's disease. Twelve of the 19 patients with ulcerative colitis were classified as pancolitis and seven had left sided disease. In the group as a whole, duration of disease ranged from 4 months to 26 years with a mean duration of 6.3 years; seven patients were diagnosed before age 21 and four before age 15. All but 10 patients were receiving 5-aminosalicylate (5-ASA) products at the time of the study. Fourteen patients were receiving local 5-ASA therapy in the form of suppositories or enemas.

6-TG METABOLITE LEVELS AND CLINICAL STATUS

At the time of the study, a history and physical examination were performed. Duration, dose, and side effects of antimetabolite therapy were

recorded for each patient. Sixty four patients were receiving AZA and 18 6-MP therapy, and this remained consistent. In patients with Crohn's disease, disease activity was measured by the Harvey-Bradshaw index (HBI). HBI <5 is considered comparable with a Crohn's disease activity index of <150, a widely used indicator of clinical remission.[18] In patients in whom the therapeutic goal was corticosteroid sparing, remission was defined as HBI <5, and clinical stability for at least one month off all corticosteroids or weaned to a low alternate day dose (20 mg on alternate days). Oral ileal released budesonide, 3 mg every other morning, was also accepted in the definition of disease remission. Either prednisone or oral budesonide therapy was tapered weekly according to the patient's clinical status. A flexible weaning schedule was used in the event of symptomatic disease recurrence as antimetabolite therapy was being instituted.[19] Patients whose therapeutic goal was closure of draining fistulas were considered in remission if all fistulas had stopped draining for at least one month. For patients with ulcerative colitis, a clinical activity score was adapted from the cyclosporin study of Lichtiger and colleagues.[20] Patients who did not meet the remission criteria were considered incompletely responsive to that dosage of antimetabolite therapy. All patients were informed of the potential risks and benefits of antimetabolite therapy prior to their agreeing to receive therapy or having their erythrocyte 6-TG metabolites measured. TPMT genotype testing has not been shown to be useful clinically in predicting clinical responsiveness to therapy, and thus was not monitored in the study group.[21]

OPTIMISATION OF THERAPY

Twenty two of 30 patients with Crohn's disease who were considered incompletely responsive to moderate doses of AZA therapy were recruited based on an intent to treat basis to study the role of optimising dosages to improve clinical responsiveness. Twelve patients had ileocolitis and 10 had colitis. Inclusion criteria included active disease, as defined by HBI >5 or draining fistulas, dependancy on corticosteroids with an erythrocyte 6-TG level <250 pmol/8×10^8RBCs, and a white blood cell count (WBC) >5.0×10^3/μl. In eight patients with refractory disease who were not included in the study, five had high erythrocyte 6-TG levels (>250 pmol/8×10^8RBCs) and went on to receive either adjunct immunomodulatory therapy or surgery. Three patients with refractory disease and low 6-TG levels (<250 pmol/8×10^8RBCs) underwent surgery and were not entered into the dose optimisation study.

The AZA dose was increased by 25 mg/day at eight week intervals if needed. Clinical status, serial WBCs, and erythrocyte 6-TG levels were monitored at each clinic visit. AZA dose was not increased if WBC was <5.0×10^3/μl. Drug induced leucopenia was defined as WBC <5.0×10^3/μl. AZA dose was lowered if leucopenia was noted.

ERYTHROCYTE 6-MP AND AZA METABOLITE LEVELS

Erythrocyte 6-MP and AZA metabolite levels were measured by reverse phase HPLC, as previously described. Concentrations of the various thiobases were expressed as pmol/8×10^8 RBCs (SEM).

STATISTICAL METHODS

χ^2 analysis was used to correlate the frequency of clinical response based on erythrocyte 6-TG levels >250 pmol/8×10^8 RBCs. Total WBCs were compared before and after AZA dosage modification using the Student's *t* test.

Results

At the time of correlation of AZA metabolite levels with clinical status, all 82 patients had been receiving AZA or 6-MP for at least 12 weeks. Sixty four of the 82 patients were receiving AZA therapy at an average dose of 1.44 (0.09) mg/kg/day. Eighteen were receiving 6-MP therapy at an average dose of 1.16 (0.09) mg/kg/day. Thirty three of 63 patients with Crohn's disease and 14 of 19 with ulcerative colitis were considered in clinical remission at the time their erythrocyte 6-TG metabolite levels were measured. Conversely, 30 patients with Crohn's disease and five with ulcerative colitis were considered incompletely responsive to antimetabolite therapy.

6-THIOGUANINE METABOLITE LEVELS

The median (range) erythrocyte 6-TG level for the entire study population receiving AZA or 6-MP therapy was 237 (40–1023) pmol/8×10^8 RBCs. Non-compliance was confirmed in four patients (6-TG <75 pmol/8×10^8RBCs) with active disease. The median (range) erythrocyte 6-TG level for patients with Crohn's disease in remission was 316 (67–1023) pmol/8×10^8 RBCs. Thirty one of 45 patients in clinical remission had erythrocyte 6-TG levels >250 pmol/8×10^8 RBCs. The median (range) erythrocyte 6-TG level for patients with incompletely responsive disease was 176 (40–488) pmol/8×10^8 RBCs. Only five of 37 patients with unresponsive IBD had 6-TG levels >250 pmol/8×10^8 RBCs ($p<0.05$).

The difference in erythrocyte 6-TG levels between patients in remission and those who were incompletely responsive to antimetabolite therapy was most significant in the 26 patients with colonic Crohn's disease (table 1). Twelve of 15 patients in remission had 6-TG levels >250 pmol/8×10^8 RBCs. In comparison, only one of 11 patients with erythrocyte 6-TG levels >250 pmol/8×10^8 RBCs had unresponsive disease. This same pattern was seen in eight patients with perianal fistulas. Only two patients had fistula closure and both had levels >250 pmol/8×10^8 RBCs. In the 29 patients with ileocolonic disease, 15 of whom had previously undergone bowel resection, there was no difference in erythrocyte 6-TG levels in those in remission or still symptomatic.

In patients with ulcerative colitis, the median 6-TG level in the 14 patients in remission was 280 pmol/8×10^8 RBCs. Eleven of 14 had

Table 1 6-Thioguanine (6-TG) metabolite levels: correlation with clinical efficacy

		6-TG >250 pmol/8×10⁸ RBCs		
Disease	*n*	*Remission*	*Non-remission*	*p Value*
Fistulising/Crohn's	8	2/2	0/6	<0.01
Colonic Crohn's	26	12/15	1/11	<0.01
Ileocolonic Crohn's	29	6/16	2/13	NS
All Crohn's	63	20/33	3/30	<0.01
Ulcerative colitis	19	11/14	2/5	NS
All	82	31/45	5/37	<0.01

erythrocyte 6-TG levels >250 pmol/8×10^8 RBCs (NS). There was no difference between patients with left sided disease or pancolitis.

6-METHYL MERCAPTOPURINE METABOLITE LEVELS

The median (range) erythrocyte 6-MMP level for the entire patient population was 2000 (668–4578). Erythrocyte 6-TG levels did not correlate with 6-MMP metabolite levels. No patient had absent 6-MMP metabolite levels.

OPTIMISATION THERAPY

The daily dosage of AZA was increased by 25 mg in 22 patients with Crohn's disease unresponsive to the starting dose of therapy. Twelve had ileocolitis and 10 had colitis. The mean (SEM) AZA dose was increased from 1.1 (0.1) to 1.5 (0.1) mg/kg/day. Eighteen patients went into remission with the increase in AZA dose. The dosage of prednisone was decreased from 18.2 (3.5) to 11.0 (2.5) mg/day. Erythrocyte 6-TG levels also increased with the modification in AZA dose from 194 (67–688) to 303 (67–737) pmol/8×10^8 RBCs. High erythrocyte 6-TG levels were achieved (>250 pmol/8×10^8 RBCs) in 12 of 18 patients who responded favourably to the adjustment in AZA dose ($p<0.05$).

Drug induced leucopenia (<3×10^3/ml) did not occur in any of the 22 patients in the dose optimisation study. The average (SEM) leucocyte count decreased from 8.6 (0.9) to 6.9 (0.6) ×10^3/ml. In four patients with persistently active disease, two had low erythrocyte 6-TG levels (<250 pmol/8×10^8 RBCs); one required surgery for intestinal obstruction and the other responded well to cyclosporin therapy. The remaining two patients with active disease and high 6-TG levels (>350 pmol/8×10^8 RBCs) went on to receive Infliximab therapy which produced a symptomatic response.

COMPLICATIONS

There were no adverse events related to optimisation of AZA therapy. Among the 82 patients surveyed in the correlation of AZA metabolite levels and clinical status, there were four adverse events. One patient had pancreatitis and one had hepatitis with markedly elevated serum aminotransferase levels (>2000 U/l). Two patients had significant leucopenia (<3 ×10^3/ml). One of these two patients had a five year history of home parenteral nutrition and died of overwhelming fungal sepsis and disseminated intravascular coagulation. All four patients had high erythrocyte 6-TG levels (>500 pmol/8×10^8 RBCs). Three of the four patients recovered completely with cessation of therapy.

Discussion

Many management decisions in patients with Crohn's disease are based on patient symptoms. Controlled trials of most medications utilise some form of activity index based almost entirely on patient complaints. If a patient has had previous surgery or has multiple symptoms and is receiving antimetabolite therapy to maintain remission, it may be difficult to know if the patient is receiving an adequate dose. In the case of AZA and 6-MP, the physician is trying to strike a balance between an adequate dose and not inducing adverse effects such as leucopenia. In comparison, neurologists who care for patients with seizures are guided by routine measurements of plasma anticonvulsant levels during therapy. In gastroenterology, similar information could be helpful in some patients who are receiving immunomodulatory therapy.

HPLC measurement of the active AZA metabolite 6-TG correlated well with clinical responsiveness to therapy in our patients with Crohn's colitis and fistulising disease but not in either ileocolonic disease or ulcerative colitis. Two thirds of patients who responded to immunomodulatory therapy had erythrocyte 6-TG levels >250 pmol/8×10^8 RBCs compared with only five of 37 patients who were still symptomatic despite prolonged therapy. Similar results have been reported in 93 paediatric and 45 adult patients with IBD in whom disease remission correlated well with erythrocyte 6-TG levels of more than 230 and 260 pmol/8×10^8 RBCs, respectively.[21 22] Interestingly, erythrocyte 6-TG levels did not correlate with clinical responsiveness to therapy in patients with ileocolonic Crohn's disease and ulcerative colitis. It was evident at the time of the study that 15 of 29 patients with ileocolonic disease had previously undergone surgery and were therefore on prophylactic AZA therapy. Indeed, 12 of 16 patients in disease remission had surgery and remained in disease remission with subtherapeutic erythrocyte 6-TG levels. In ulcerative colitis, mesalamine has proven efficacy in the management of mild to moderate disease.[23] Our aggressive use of local 5-ASA preparations in 14 of 19 patients with ulcerative colitis may explain why there was a lack of correlation between clinical response and erythrocyte AZA metabolite levels. These studies indicate the putative immunosuppressive role of 6-TG in patients with IBD, and support the idea of using erythrocyte 6-TG levels to develop a therapeutic index. Lack of compliance with therapy can also be detected.

If erythrocyte 6-TG levels are less than 250 pmol/8×10^8 RBCs in a symptomatic patient who has been receiving long term AZA therapy (>12 weeks) and has a normal WBC (>5 ×10^3/ml), one reasonable option would be to increase the dosage by 25 mg with the goal of achieving a more complete therapeutic response over the next eight weeks. In addition, the potential for under dosing AZA as a maintenance therapy after cyclosporin or Infliximab induced remission or as prophylactic therapy in patients after surgery is evident in this study. As

TPMT enzyme activity levels were not available, low dosages of either AZA or 6-MP were used to minimise potential drug induced toxicity. It was therefore not surprising that 32 of 37 patients in non-remission had subtherapeutic erythrocyte 6-TG levels. The study would suggest that the physicians' concerns with under dosing can be circumvented by measuring erythrocyte 6-TG levels, as described below.

In the prospective study of 22 patients with steroid dependant Crohn's disease, treatment efficacy correlated with improved erythrocyte 6-TG metabolite levels following an adjustment in antimetabolite dosage. All patients were initially described as unresponsive to therapy despite receiving AZA at a dosage of 1–1.2 mg/kg/day for at least 12 weeks. In 18 of 22 patients recruited into the study, clinical remission was achieved by optimising the dose of AZA therapy and without inducing leucopenia.

This current study and our previous paediatric study would suggest that measurement of erythrocyte 6-TG levels can be used to optimise the dose of AZA to achieve a desired therapeutic effect while avoiding untoward drug toxicity.[24] Patients who remain symptomatic despite apparently therapeutic erythrocyte 6-TG levels could be expected to receive other forms of therapy, including cyclosporin, methotrexate, Infliximab, or surgery.

Lowering of WBC can also be considered an indication that therapeutic AZA metabolite levels have been achieved in that individual. Colonna and Korelitz have shown that with 1 mg/kg of 6-MP, there was a more rapid (<7 weeks) and more complete clinical response in patients in whom leucopenia ($<5\times10^3$/ml) developed compared with those who did not have a lowering of their WBC below 5×10^3/ml. The average response in the latter group was 13 weeks.[25] Presumably, these differences in response time to the same dose of 6-MP reflect, to a large extent, differences in 6-MP bioavailability or metabolism.[26] Unfortunately, some clinicians have misinterpreted this important paper as suggesting that a patient should not be considered refractory to 6-MP therapy until the dosage has been increased to produce leucopenia. Instead, we would suggest the use of erythrocyte 6-TG levels when trying to optimise antimetabolite therapy in the patient with symptoms or fistulae seemingly refractory to the effects of these agents. In our current study, remission was obtained in 18 of 22 patients by increasing the dose until the presumed therapeutic range was reached. None of the 18 had WBCs less than 5×10^3/ml.

1 Pearson DC, May GR, Fick GH, *et al*. Azathioprine and 6-mercaptopurine in Crohn's disease: a meta-analysis. *Ann Intern Med* 1995;**122**:132–42.
2 O'Brien JJ, Bayless TM, Bayless JA. Use of azathioprine or 6-mercaptopurine in the treatment of Crohn's disease. *Gastroenterology* 1991;**101**:39–46.
3 Ewe K, Press AG, Singe CC, *et al*. Azathioprine combined with prednisolone or monotherapy with prednisolone in active Crohn's disease. *Gastroenterology* 1993;**105**:367–72.
4 Korelitz BI, Adler DJ, Mendelsohn RA, *et al*. Long-term experience with 6-mercaptopurine in the treatment of Crohn's disease. *Am J Gastroenterol* 1993;**88**:1198–205.
5 Present DH, Korelitz BI, Wisch N. Treatment of Crohn's disease with 6-mercaptopurine. A long-term, randomized, double-blind study. *N Engl J Med* 1989;**302**:981–7.
6 Christie NT, Drake S, Meyn RE. 6-Thioguanine induced DNA damage as a determinant of cytotoxicity in cultured hamster ovary cells. *Cancer Res* 1986;**44**:3665–71.
7 Lennard L. The clinical pharmacology of 6-mercaptopurine in acute lymphoblastic leukemia. *Eur J Clin Pharmacol* 1992;**43**:329–39.
8 Elion GB. The pharmacology of azathioprine. *Ann NY Acad Sci* 1977;**21**:401–7.
9 Van Os EC, Zigs BJ, Sandborn WE, *et al*. Azathioprine pharmacokinetics after intravenous, oral, delayed release oral and rectal foam administration. *Gut* 1996;**39**:63–8.
10 Bostrom B, Erdman G. Cellular pharmacology of 6-mercaptopurine in acute lymphoblastic leukemia. *Am J Pediatr Hematol Oncol* 1993;**15**:80–6.
11 Fairchild CR, Maybaum J, Kennedy KA. Concurrent unilateral chromatid damage and DNA strand breaks in response to 6-thioguanine treatment. *Biochem Pharmacol* 1986;**35**:3533–41.
12 Lennard L, Lilleyman JS. Variable mercaptopurine metabolism and treatment outcome in childhood lymphoblastic leukemia. *J Clin Oncol* 1989;**7**:1816–23.
13 Evans WE, Homer M, Chu YQ. Altered mercaptopurine metabolism, toxic effects, and dosage requirements in a thiopurine methyl transferase deficient child with acute lymphoblastic leukemia. *J Pediatr* 1991;**119**:985–9.
14 Sandborn WJ, Tremaine WJ, Wolfe DC, *et al*. Lack of effect of intravenous administration on time to respond to azathioprine for steroid-treated Crohn's disease. *Gastroenterology* 1999;**117**:527–35.
15 Present DH, Meltzer SJ, Krumholz MP, *et al*. 6-mercaptopurine in the management of inflammatory bowel disease: short and long-term toxicity. *Ann Intern Med* 1995;**111**:641–9.
16 Cuffari C, Theoret Y, Latour S, *et al*. 6-mercaptopurine metabolism in Crohn's disease correlation with efficacy and toxicity. *Gut* 1996;**39**:401–6.
17 Kombluth A, Solomon P, Sachar DB. Crohn's disease. In: Sleisenger MH, Fordtran JS, editors. *Gastrointestinal diseases*. Philadelphia: WB Saunders, 1993:1270–304.
18 Yashida EM. The Crohn's disease activity index, its derivatives and the inflammatory bowel disease questionnaire: a review of instruments to assess Crohn's disease. *Can J Gastroenterol* 1999; **13**:65–73.
19 Malchow H, Ewe K, Brandes JW. European Cooperative Crohn's disease study (ECCDS): results of drug treatment. *Gastroenterology* 1984;**86**:249–66.
20 Lichtiger S, Present D, Kombluth A. Cyclosporin in severe ulcerative colitis refractory to steroid therapy. *New Engl J Med* 1994;**330**:1841–5.
21 Dubinsky MC, Lamothe S, Yang HY. Optimizing and individualizing 6-MP therapy in IBD: The role of 6-MP metabolite levels and TPMT genotyping. *Gastroenterology* 2000;**118**;705–13.
22 Achar JP, Stevens T, Brzezinski A, *et al*. 6-thioguanine levels versus white blood cell counts in guiding 6-mercaptopurine and azathioprine therapy. *Am J Gastroenterol* 2000;**95**: A272.
23 Hanauer S, Schwartz J, Robinson M, *et al*. Mesalamine capsules for the treatment of active ulcerative colitis. Results of a controlled trial. *Am J Gastroenterol* 1993;**88**:1188–97.
24 Cuffari C, Sharma S. 6-mercaptopurine metabolites tailored to achieve clinical responsiveness in paediatric IBD. *Gastroenterology* 1999;**116**:A694.
25 Colonna T, Korelitz B. The role of leukopenia in 6-mercaptopurine-induced remission of refractory Crohn's disease. *Am J Gastroenterol* 1993;**89**:362–6.
26 Zimm S, Collins JM, Riccardi R. Variable bioavailability of oral mercaptopurine. Is maintenance chemotherapy in acute lymphoblastic leukemia being optimally delivered. *N Engl J Med* 1983;**308**:1005–9.