European Review for Medical and Pharmacological Sciences 2014; 18: 165-170

Azathioprine treatment in inflammatory bowel disease patients: type and time of onset of side effects

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Abstract. – BACKGROUND: Azathioprine (AZA) and 6-mercaptopurine (6-MP), purine analogues, are the immunosuppressant drugs most frequently used for inducing and maintaining remission in inflammatory bowel disease (IBD). The occurrence of adverse effects is a major drawback in the use of these drugs, and short- and long-term toxicity represent a major limitation to their use.

AIM: The present study investigated the prevalence, type and time of onset of AZA-related adverse events, in a cohort of IBD patients in a single referral Centre.

PATIENTS AND METHODS: The records of consecutive IBD outpatients, referred to our Institution between 1987-2009, were retrospectively evaluated.

RESULTS: We reviewed 2014 patients, in whom AZA was prescribed in 302 of them, 139 (46%) with ulcerative colitis (UC) and 163 (54%) with Crohn's disease (CD). Side-effects were complained by 98 (32.4%) out of 302 patients, 50 UC and 48 CD, (36% UC vs 29.4% CD, $p = 0.26$). In 20 (20.4%) patients, 11 UC and 9 CD, side-effects recovered after dosage reduction whilst in 78 (79.6%), 39 UC and 39 CD, the treatment was discontinued (dose-dependent side-effects in 42 patients and dose-independent in 36). Overall, side-effects were observed after a mean period of 14.5±7.8 months (range 0.5-123) of AZA treatment. The majority (76%) of the dose-dependent adverse events were reported between 12-18 months after the beginning of treatment.

CONCLUSIONS: The prevalence of side effects leading to withdrawal of AZA treatment, in our series of Italian patients, was higher respect to data reported in the literature (25.8%).

Key Words:
Azathioprine, Side-effects, Ulcerative colitis, Crohn's Disease.

Introduction

Purine analogues, azathioprine (AZA) and 6-mercaptopurine (6-MP), are the immunosuppressant drugs most frequently used for inducing and maintaining remission in inflammatory bowel disease (IBD)[1-3].

The occurrence of adverse effects is a major drawback in the use of these drugs, and short- and long-term toxicity represent a major limitation to their use[4-6], leading to withdrawal of treatment in a variable proportion of patients. The prevalence of adverse effects reported in literature ranges between 5-6% and 30% of patients[7-16].

The side-effects of thiopurine treatment are classified as "dose-dependent" (myelotoxicity, hepatitis, cancer, opportunistic infections) and "dose-independent", this group comprising a range of side-effects resulting from allergic (rash, fever, arthralgia) and idiosyncratic reactions (myelotoxicity, pancreatitis, hepatitis)[10,17,18]. Dose-dependent adverse effects are triggered by the production and accumulation of toxic metabolites, due to the ineffective intracellular metabolic pathways involved in drug metabolism and disposal.

Irrespective of the variable frequency of adverse events requiring withdrawal from treatment, in the various series, those most commonly reported are: drug allergy, leukopenia, pancreatitis and nausea[5,6,9-14]. Mild leukopenia is a relatively common haematological adverse effect, but severe myelosuppression resulting in thrombocytopenia and, less frequently, pancytopenia have also been reported. Potentially lethal severe bone marrow suppression occurs in 2-5% of patients treated with AZA or 6-MP[19].

Aim of this study was to investigate the prevalence, type and time of onset of AZA-related adverse effects in a cohort of IBD patients observed in a single Italian Centre.

Patients and Methods

Data from 2014 IBD patients treated in our Institution, between 1987-2009, were reviewed. All

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consecutive patients treated with AZA were included in this retrospective study. AZA was administered at the recommended dose of 2-2.5 mg/kg.

Diagnosis of Crohn's disease (CD) and ulcerative colitis (UC) were established according to standard clinical, radiological, histological and endoscopic criteria. The Vienna classification of CD[20], based on age at diagnosis (< 40 yr [A1], ≥40 yr [A2]), location (terminal ileum [L1], colon [L2], ileocolon [L3], upper gastrointestinal tract [L4]), and behaviour (non-stricturing non-penetrating [B1], stricturing [B2], penetrating [B3]), was used. The classification used for UC patients was based on the location and extension of the disease: proctitis/proctosigmoiditis, left-side colitis (up to the splenic flexure), extensive colitis (up to the hepatic flexure), and pancolitis.

Blood chemistry was analyzed before administration of the drug and thereafter every 10-15 days for the first 3 months and then every 2-3 months after the onset of treatment.

Indications for treatment were defined as follows:

1. "Steroid-dependent" disease: patients who are either unable to reduce steroids below the equivalent of prednisolone 10 mg/day (or budesonide below 3 mg/day) within 3 months of starting steroids, without recurrent active disease, or who have a relapse within 3 months of stopping steroids[21];
2. "Steroid-refractory" disease: patients who have active disease despite prednisolone of up to 0.75 mg/kg/day over a period of 4 weeks[21];
3. "Penetrating" CD: presence of fistulae;
4. "Extra-intestinal" manifestations (EIMs).

Patients were considered as responders when AZA was effective in inducing or maintaining remission, with a steroid-sparing effect or as intolerant when the occurrence of side-effects required withdrawal from treatment or reduced dosage. Patients were considered as "non- responders" when treatment failed to induce or maintain remission.

The main side-effects were classified in "dose-independent" such as allergic (rash, fever, arthralgia) and idiosyncratic reactions (myelotoxicity, pancreatitis, hepatitis) and in "dose-dependent" side-effects (myelotoxicity, hepatitis, cancer, opportunistic infections)[10,17,18].

Myelotoxicity was defined according to the criteria used by Connell et al (18) as either leukopenia (white blood count < $3.0 \cdot 10^9/l$) and/or thrombocytopenia (platelet count < $100.00 \cdot 10^6/l$) resolving after withdrawal of treatment or dose reduction. Hepatotoxicity was defined as an increase in serum alanine transaminase greater than twice the upper normal limit and as resolution after withdrawal of treatment or reduction in the dosage. Pancreatitis was defined as upper abdominal pain with pancreatic amylase and lipase blood levels more than twice the normal upper limit.

Statistical Analysis

For the statistical analysis, the Fischer's Exact test (including Yates' correction) for categorical data was used. Probability values and confidence intervals (CI) were calculated at the 95% level. Differences were considered significant when $p \leq 0.05$ was reached.

Results

Azathioprine has been prescribed in 302 patients (15%), 163 (54%) with CD and 139 (46%) with UC. Of these, 164 (54.3%) were males. Clinical and demographic characteristics of the patients enrolled are outlined in Table I.

Side-effects were complained by 98 (32.4%) out of 302 patients, 50 with UC and 48 with CD with a prevalence of 36% and 29.4% respectively ($p = 0.26$). Adverse effects were observed after a mean period of 14.5±7.8 SD months (range 0.5-123 months, median 4.5 months).

Overall, mielotoxicity was observed in 50 (16.6%) patients (29 UC vs 21 CD); pancreatitis in 13 (4.3%) (3 UC vs 10 CD), hepatitis in 10 (3.3%) (7 UC vs 3 CD), vomiting in 6 (2%) (2 UC vs 4 CD), nausea in 7 (2.3%) (3 UC vs 4 CD), allergic reaction in 4 (1.3%) (2 UC vs 2 CD), urticaria in 3 (1%) (2 UC vs 1 CD), 1 case of arthralgia (0.3%) and thrombocytopenia (0.3%) in UC and 1 case of lymphoma (0.3%), erythema nodosum (0.3%) and renal toxicity (0.3%) in CD, respectively. No significant statistically difference were observed between these two disease (Table II). In 78 (25.8%) of these patients, 39 with UC and 39 with CD, the treatment was discontinued while in other 20 (6.6%) the side-effects recovered after dosage reduction (Tables III-IV). One patient died from broncopneumonia associated with severe leukopenia. A total of 137 patients (45.4%) are still under treatment with AZA. Overall 67 pa-

Table I. Clinical and demographic characteristics of IBD (302) patients treated with AZA.

	Crohn's disease n. =163	Ulcerative colitis n. =139
Males/Females	92/71	72/67
Mean age years at diagnosis ±S.D.	31.95 ± 13.01	33.15 ± 13.73
Location/extension, n		
Ileum	50	–
Ileum + colon	78	–
Colon	15	–
Upper GI	20	–
Pancolitis	–	85
Left-sided colitis	–	31
Procto-sigmoiditis	–	23
Behaviour, n		
Non-stricturing, Non-penetrating	50	–
Penetrating	36	–
Stricturing	77	–

Table II. Total side effects of AZA treatment in ulcerative colitis (50) and Crohn's disease (48) patients.

Side-effects	n.	Ulcerative colitis	Crohn's disease
Leukopenia	50	29	21
Pancreatitis	13	3	10
Hepatitis	10	7	3
Vomiting	6	2	4
Nausea	7	3	4
Allergic reaction	4	2	2
Urticaria	3	2	1
Arthralgia	1	1	0
Erythema nodosum	1	0	1
Lymphoma	1	0	1
Thrombocytopenia	1	1	0
Renal toxicity	1	0	1
Total	**98**	**50**	**48**

tients (22.2%) discontinued treatment, 43 (14.2%) after prolonged remission and 24 (8%) due to treatment failure.

The seventy-eight patients with withdrawal of treatment presented dose-dependent side effects in 42 patients (19 with UC and 23 with CD) and dose-independent in 36 (20 with UC and 16 with CD). Dose-dependent side-effects occurred in 76% (32/42) of patients between 12-18 months after the beginning of the treatment.

Table III. Dose-independent side-effects in ulcerative colitis (20) and Crohn's disease (16) patients with withdrawal of treatment.

Side-effects	n.	Ulcerative colitis	Crohn's disease
Leukopenia	16	11	5
Vomiting	6	2	4
Pancreatitis	5	2	3
Allergic reaction	4	2	2
Urticaria	3	2	1
Arthralgia	1	1	0
Erythema nodosum	1	0	1
Total	**36**	**20**	**16**

Table IV. Dose-dependent side-effects in ulcerative colitis (19) and Crohn's disease (23) patients with withdrawal of treatment.

Side-effects	n.	Ulcerative colitis	Crohn's disease
Leukopenia	16	8	8
Hepatitis	10	7	3
Pancreatitis	8	1	7
Nausea	5	2	3
Lymphoma	1	0	1
Thrombocytopenia	1	1	0
Renal toxicity	1	0	1
Total	**42**	**19**	**23**

Discussion

AZA and its active metabolite 6-MP have been demonstrated to be an effective therapeutic tool in the treatment of IBD patients over the last 40 years. Their use, however, is limited by the occurrence of adverse effects reported in 5-30% of patients[7,8,16,22]. The variable prevalence reported in the literature may be due to various factors, ranging from the genetic background of the population studied to the non-univocal definition of the single side-effects.

The main genetically-controlled factor is considered to be the thiopurine S-methyltransferase enzyme (TPMT), responsible for AZA s-methylation, which is the predominant inactivation pathway in haematopoietic tissues. The genetic polymorphism of TPMT affects the biological activity. Patients with genetically determined TPMT deficiency accumulate high levels of active thioguanine nucleotides following the use of standard doses of the drug[23]. Studies on North-American and West-European populations showed that approximately 90% of individuals inherit high (normal) TPMT activity, 10% intermediate activity due to heterozygosity, and 0.3% low or no detectable activity resulting from two non-functional TPMT alleles. A number of TPMT gene mutations have been reported in the literature, involving at least 70 TPMT alleles. The most prevalent, TPMT are * 2, * 3A, * 3C accounting for 80-95% of all cases with intermediate or low enzyme activity[24]. However, this does not always occurs, in fact, in some populations a large proportion of intermediate methylators are not associated with known TPMT alleles[25,26]. Moreover, in some countries, including Spain, France and other mediterranean countries, as well as in the Jewish population, TPMT activity follows an unimodal distribution pattern unlike the trimodal pattern reported in other areas[27]. Thus, the study of TPMT expression proves useful in predicting the occurrence of dose-dependent side-effects only in a relatively small proportion of patients. The cost effectiveness of its widespread clinical use remains to be defined[5]. In the present series, in according to the ECCO (European Crohn's and Colitis Organization) guidelines, TPMT activity was not taken into consideration in fact, no recommendation can be made about routine measurement of TPMT activity or genotype prior to initiating thiopurine therapy[28].

Another important factor determining the variable prevalence of adverse effects, reported in the different series, is related to their definitions. Granulocytopenia is usually, but not invariably, defined as a reduction below $3.0 \cdot 10^9/l$. On the other hand, the threshold values of transferases, lipase and amylase are not clearly defined and, in most instances, this crucial information is not provided by the Authors[29,30].

Moreover, since standard dose of AZA is defined in the various Institutions after an induction phase ranging from a few days to several weeks, the time required for the occurrence of dose-dependent side-effects varies considerably.

Finally, the prevalence of the observed side-effects is related to the scheduled controls of laboratory data. Performing laboratory analyses every 2-3 months, or discontinuing them after 6-9 months, may, indeed, lead to an underestimation of the prevalence of adverse effects. This is particularly true when abnormal laboratory data are not associated with clinical symptoms.

Conclusions

In the series of patients included in the present investigation, the prevalence of adverse effects leading to withdrawal of AZA treatment was

high (25.8%), and in a further 6.6% of patients, reduction of AZA below the recommended dose was also necessary. Interestingly, 76% of dose-dependent side-effects were observed between 12-18 months after the beginning of treatment. The reason for this finding is unclear. The genetic background of the population studied could be a possible explanation. Alternatively, a less than optimal compliance to drug treatment could have played a role in the late occurrence of dose-dependent adverse effects. Irrespective of the cause, in our opinion, this represents the most relevant finding of the present study, since laboratory data are usually evaluated only during the first 3-6 months of treatment. Thus, the need to extend the laboratory work-up for 12-18 months after the onset of AZA treatment is strongly supported by the present data.

Conclusions

Early identification of adverse effects, leading to a dosage reduction or even complete withdrawal of the drug, would prevent a considerable proportion of severe complications.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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