Chapter VI

Safety of high-dose azathioprine in immunobullous patients

Summary
The most important side-effects of high-dose azathioprine are myelosuppression and liver toxicity. Since the arrival of the thiopurine methyltransferase (TPMT) assay, pancytopenia can be predicted in those patients with low enzyme levels. This also opened the opportunity to prescribe higher doses of azathioprine in the patients with normal TPMT levels. We reviewed the sequelae of 14 patients with immunobullous disease treated with high-dose azathioprine (2-3 mg/kg/day), and followed the blood cell counts and liver enzyme levels. From this study we conclude that high-dose azathioprine can safely be used in immunobullous conditions and that the assay may guard the safety of this schedule.
Introduction

Azathioprine is for over 30 years widely used as adjuvant immunosuppressive agent to systemic corticosteroids in a variety of dermatologic conditions (1-3). Apparently good clinical response to the combination of azathioprine and prednisone was reported in pemphigus (4;5) bullous pemphigoid (6), and mucous membrane pemphigoid (7). In a randomized, not blinded, trial in bullous pemphigoid, Burton et al. found a 45% steroid-sparing effect with high dose azathioprine (2.5 mg/kg) (8). In another randomized, not blinded, trial in bullous pemphigoid, Guillaume et al. found no steroid-sparing effect with low dose azathioprine (100-150 mg) (9). Randomized placebo-controlled trials evaluating the steroid-sparing effect of azathioprine in immunobullous disease are so far lacking in the literature (10). Nevertheless, there is a communis opinio that azathioprine is first choice adjuvant in the treatment of autoimmune bullous diseases such as pemphigus, because of the low toxicity/effect ratio (1;11). The most frequent reasons that make azathioprine dose reduction necessary are myelosuppression and liver toxicity. Common practice is to prescribe low dose (50-150 mg) or to adjust azathioprine dose according to body weight only, and not to individual drug metabolism. However, the metabolism of azathioprine is individually variable and not related to the body weight (6).

In 1980 Weinshilboum and Sladek (12) revealed the pharmacokinetics of mercaptopurine, the active metabolite of azathioprine. The purine antagonist azathioprine, is rapidly absorbed and methylated in the intestine to 6-mercaptopurine (6-MP), which is then metabolized in the liver and erythrocytes via three competitive pathways. The hypoxanthine phosphoribosyl transferase pathway produces several metabolites including 6-thioguanine nucleotides (6-TGNs), which are responsible for suppression of de novo purine synthesis and cytotoxicity. The thiopurine methyltransferase (TPMT) activity and xanthine oxidase pathways produce inactive metabolites which are excreted in the urine. TPMT shows wide interindividual variation, but xanthine oxidase does not (6). The TPMT pathway thus determines the drug clearance.

The active metabolites of azathioprine may evoke leukopenia, occurring in 5-25% of all patients (6). In particular, patients with low or intermediate TPMT activity are
vulnerable for this hematological side-effect. Allelic polymorphism’s of the TPMT gene determine the activity of the coded enzyme. Homozygotes (1 in 300) for the low activity allele are known to be at serious risk for acute myelosuppression after azathioprine intake (12). Heterozygotes with intermediate TPMT enzyme activity (11% of the patients), may be at risk for late-onset myelosuppression (6). In contrast, homozygotes for the high activity allele may be suboptimal immunosuppressed with the common standard dose of azathioprine. The TPMT profile can be assessed either by genotyping DNA or by phenotyping measuring TPMT enzyme activity in erythrocytes.

In this study we assessed the safety in terms of myelosuppression and liver toxicity of high-dose azathioprine, up to 3 mg/kg dd, in out-patient dermatological practice. The calibration of the new TPMT enzyme activity assay was determined in 152 Dutch controls. Subsequently, we determined the TPMT enzyme level and genotype on a series of immunobullous patients.

Patients and methods

14 patients with immunobullous disease were included who were treated with azathioprine and prednisone within the period 1998-2001. Azathioprine was started with a test dose of 50 mg for three days, so that the drug could be easily withdrawn at early stage if gastro-intestinal complaints would occur. Subsequently the azathioprine dose was increased to 2-3 mg/kg per day. Azathioprine dose was not tapered at the end of the treatment period and continued for 3-12 months as monotherapy after prednisone was tapered to zero.

We used the following guidelines for dose reduction in case of myelosuppression or liver toxicity. Daily dosage azathioprine was halved or reduced with 1 mg/kg in case of leukopenia of less than 4x10⁹/L, and stopped when leukopenia was less than 2x10⁹/L, or when trombocytopenia was less than 100x10⁹/L. In case the liver enzymes raised twice above high-normal value daily azathioprine dose was reduced with 50 mg and after two weeks evaluated again, and if necessary again tapered with 50 mg until the liver enzymes reached normal values. All patients received concomitant prednisolone.

Since measurement of both TPMT enzyme activity in erythrocytes and TPMT genotyping have only recently become available in our hospital, all TPMT tests were
performed after the start of the azathioprine medication. TPMT activity in erythrocytes was determined in EDTA-anticoagulated venous blood samples by HPLC-fluorometry essentially as described the group of Iven and coworkers (13;14). Hemoglobin content of cell lysates was determined with a Coulter STKS (Coulter Corporation). We used 6-ethylmercaptopurine (6-EMP) (Sigma, Holland) as an internal standard to correct for extraction losses during the extraction procedure. TPMT erythrocyte activity reference values were determined in 152 samples offered for routine haemocytometric analysis from adult patients.

Genotyping was performed as described by Yates et al (15). Polymerase chain reactions were performed to detect the G238C transversion (exon 5), the G460A (exon 7) and A719G (exon 10) transitions. Laboratory parameters, i.e. leukocytes, thrombocytes, and liver enzymes (ASAT, ALAT, γGT, LDH), were monitored at start of therapy, after one month, and than every three months. In this study, no efforts were made to assess a clinical efficacy.

Results

Figure I shows the frequency distribution of erythrocyte thiopurine methyltransferase in 152 samples offered for routine hemocytometric analysis. In this figure a bimodal distribution can be seen. The enzyme activity in the investigated samples ranged from 19.75 to 89.20 nmol 6-methylthioguanine (6-MTG) g\textsuperscript{-1} Hb.h\textsuperscript{-1}, with a median of 49.96 nmol 6-MTG g\textsuperscript{-1} Hb.h\textsuperscript{-1}. 6-MTG, the metabolite of 6-thioguanine (6-TG) is highly fluorescent and therefore more sensitive to detect by HPLC. The break point between intermediate and high TPMT activity was set at 35 nmol 6-MTG g\textsuperscript{-1} Hb.h\textsuperscript{-1}. The breakpoint between intermediate and low was set at 5 nmol 6-MTG g\textsuperscript{-1} Hb.h\textsuperscript{-1}. We found no samples with [C] < 5 nmol 6-MTG g\textsuperscript{-1} Hb.h\textsuperscript{-1} TPMT activity.
Table I lists the included patients, diagnosis, daily dose of azathioprine, TPMT phenotype, TPMT genotype, and side-effects. Two patients (#1, #2) developed leukopenia during azathioprine treatment.

In patient #1, a leukopenia of 2.9x10⁹/L was encountered four months after the start of azathioprine 3 mg/kg per day. Daily dosage was tapered to 2 mg/kg per day, whereafter the leukocyte number increased to 4.3x10⁹/L. After knowledge of a normal TPMT enzyme activity (55.7 nmol 6-MTG g⁻¹ Hb.h⁻¹), azathioprine could be raised to 2.5 mg/kg per day without further hematological disturbances.

In patient #2, pretreatment leukocyte number was low at 4.0x10⁹/L. Two months after medication with azathioprine 2 mg/kg per day the leukocyte number decreased to 3.2x10⁹/L. Daily dosage azathioprine was stepwise reduced to 0.7 mg/kg per day until the leukocyte number was above 4.0x10⁹/L. The TPMT activity was intermediate (34.0 nmol 6-MTG g⁻¹ Hb.h⁻¹) in this patient, who was homozygous for high activity TPMT alleles.

In patients #3 and #4 daily dosage azathioprine had to be decreased because of liver toxicity. A reduction azathioprine with 50 mg per patient normalized in both patients the liver enzymes. Only one patient (#5) experienced temporary mild gastro-intestinal complaints, without necessity to stop therapy. None of the 14 patients carried low activity
TPMT alleles. Nevertheless 2 patients had a TPMT level below 35 nmol 6-MTG g\(^{-1}\) Hb.h\(^{-1}\). Taken together, an azathioprine dose of 2-3 mg/kg per day was well tolerated in 13 out of 14 patients.

**Table I. Patients on high-dose azathioprine**

<table>
<thead>
<tr>
<th>No./Age/Sex</th>
<th>Diagnosis</th>
<th>Azathioprine (mg/kg)</th>
<th>Duration therapy (months)</th>
<th>TPMT level (phenotype) (nmol 6-MTG g(^{-1}) Hb.h(^{-1}))</th>
<th>Genotype</th>
<th>TPMT alleles</th>
<th>Leukopenia (TSH)</th>
<th>Comments / adverse-effects</th>
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<tbody>
<tr>
<td>1/47/M</td>
<td>MMP</td>
<td>3</td>
<td>8</td>
<td>55.7</td>
<td>Wt/Wt</td>
<td>Y</td>
<td></td>
<td>p&lt;0.05 1.4x10(^{10})L, after 4 months azu 1.5 mg/kg per day, L 2.9x10(^{10})L, azu 4.3x10(^{10})L, after normal TPMT, azu 2.5 mg/kg per day, L 4.0x10(^{10})L, p&lt;0.05 1.4x10(^{10})L, after 2 months azu 2mg/kg per day, L 3.2x10(^{10})L, azu 1.5mg/kg per day, L 3.6x10(^{10})L, azu 1.7mg/kg per day, L 4.0x10(^{10})L</td>
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<tr>
<td>2/70/M</td>
<td>BP</td>
<td>2.5</td>
<td>36</td>
<td>34.0</td>
<td>Wt/Wt</td>
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<td>3/42/M</td>
<td>PV</td>
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<td>3</td>
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<td>Normal liverfunction at 1.5 mg/kg per day</td>
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<td>27</td>
<td>53.6</td>
<td>Wt/Wt</td>
<td>N</td>
<td></td>
<td>Elevated aat 92, abt 114, yOT 483</td>
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<td>12</td>
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<td>Normal liverfunction at 1.5 mg/kg per day</td>
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<td></td>
<td>Mild gastro-intestinal complaints</td>
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<td>11/37/F</td>
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<td>56.9</td>
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Legend: MMP = mucous membrane pemphigoid, PV = pemphigus vulgaris, PF = pemphigus foliaceus, BP = bullous pemphigoid, aza = azathioprine, Wt = wild type

**Discussion**

Azathioprine is generally well tolerated (16), but is known to cause severe myelosuppression in a small group of patients due to allelic polymorphism of the TPMT enzym activity (12). Common practice is to prescribe a mostly inadequate low dose azathioprine (50-150 mg per day) or to adjust the dose according to body weight only. High-dose azathioprine (2-3 mg/kg/day) is mostly reserved for recalcitrant patients. In transplantation medicine daily dose azathioprine goes up to 5 mg/kg/day (17). Using high-doses azathioprine, is nowadays guarded by determining TPMT enzyme activity. Even better is to monitor 6-thioguanine nucleotides (6-TGN), the toxic metabolites of azathioprine (17), during therapy.
In our small study population no homozygotes for low activity of TPMT were found. Of the two patients with phenotypical intermediate TPMT enzyme activity, only one patient experienced chronic leukopenia. Two patients had liver toxicity, disappearing after tapering daily dosage azathioprine.

The frequency distribution of erythrocyte thiopurine methyltransferase activity in our study group showed the bimodal distribution that can be expected on the basis of TPMT polymorphism frequency (89% high, 11% intermediate, and 0.3% low TPMT enzyme activity) (12;18;19). Using the same method for TPMT enzyme activity measurement, Kroplin et al. (14) found a trimodal distribution in 214 subjects. This can be explained by the difference in size of the study groups. The median TPMT enzym activity in our study group was higher than found by Kroplin et al., probably due to a correction made for extraction losses by usage of an internal standard. Dosing azathioprine may possibly be done on a constant azathioprine/TPMT activity ratio and thus further optimize the immunosuppressive effect per patient.

This study illustrates the potential clinical benefits of elucidating the molecular basis of inherited differences in drug metabolism and disposition, and makes it feasible to more precisely select the optimal drug and dosage for individual patients. High-dose azathioprine can safely be used in immunobullous conditions when TPMT-phenotyping is performed to assess the drug tolerance of the individual patient.

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References