AZATHIOPRINE-RELATED BONE MARROW TOXICITY AND LOW ACTIVITIES OF PURINE ENZYMES IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Objective. Azathioprine (AZA) metabolism largely parallels the endogenous purine pathways. To date, thiopurine methyltransferase (TPMT) deficiency has been reported as a cause of AZA-related bone marrow toxicity in 1 patient with rheumatoid arthritis (RA). We therefore studied purine enzyme activities in 3 patients with RA who experienced AZA-related bone marrow toxicity.

Methods. Lymphocyte activity of purine nucleoside phosphorylase and 5'-nucleotidase (5NT) and erythrocyte activity of TPMT, key enzymes in thiopurine catabolism, were measured in 3 RA patients who had experienced AZA-related bone marrow toxicity and in 16 RA patients without signs of toxicity despite at least 6 months of treatment with AZA.

Results. Two patients with AZA-related bone marrow toxicity were found to have a TPMT deficiency, 1 partial and 1 total. In the third patient, 5NT activity was found to be well below the lowest level observed in the control subjects.

Conclusion. All 3 patients with severe AZA-related bone marrow toxicity had abnormal purine enzyme activities. Deficiency of purine enzymes, including TPMT and 5NT, may be a cause of AZA-related bone marrow toxicity in patients with RA.

Acute, severe bone marrow toxicity is a relatively rare but potentially life-threatening side effect of azathioprine (AZA) when it is taken in dosages commonly prescribed for the treatment of rheumatoid arthritis (RA) (<3 mg/kg/day). After conversion to 6-mercaptopurine, the metabolism of AZA parallels the endogenous purine pathways (Figure 1). Low activity of thiopurine methyltransferase (TPMT) has occasionally been reported as a cause of AZA-related bone marrow toxicity in patients with a variety of conditions, including 1 with RA (1-3). However, other mechanisms must be present as well, since normal TPMT activity was found in 9 renal transplant patients with leukopenia attributed to AZA (4). To date, no cases of AZA-related bone marrow toxicity associated with abnormalities in purine enzymes have been reported in the rheumatology literature. We studied the activities of 3 key enzymes of purine catabolism in 3 patients with RA who experienced AZA-related bone marrow toxicity and in 16 control patients with RA.

PATIENTS AND METHODS

Control RA patients. Sixteen patients (9 male, 7 female) with RA (15 rheumatoid factor [RF] positive) with normal liver and kidney function, who had shown no bone marrow toxicity after at least 6 months of treatment with AZA (1-3 mg/kg/day) served as the control group.

Patients. Three patients who experienced acute AZA-related bone marrow toxicity were studied. Patient 1, a 63-year-old man with RF-positive RA since 1973, had been treated with aurothioglucose 40 mg monthly for >10 years when AZA 2.0 mg/kg daily was added to his treatment regimen because of active polyarthritis with nodules and vasculitic cutaneous ulcers. Except for slight anemia, hematologic parameters and kidney function were normal at that time. After 4 weeks, pancytopenia developed, and both AZA and aurothioglucose had to be discontinued (Table 1). Prednisone (40 mg/day) was started, and within 2 weeks, the hematologic parameters improved (hemoglobin 6.1 mmoles/liter, white blood cell [WBC] count 5.8 x 109/liter, platelet count 402 x 109/liter). At that time aurothioglucose, 50 mg

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Figure 1. Pathways in thiopurine metabolism. TPMT = thiopurine methyltransferase; Me-t = methyl-thiopurine (t = thio); 5NT = 5'-nucleotidase; HGPR = hypoxanthine-guanine-phosphoribosyltransferase; PNP = purine nucleoside phosphorylase; XO = xanthineoxidase.

Table 1. Three rheumatoid arthritis patients with azathioprine (AZA)-related bone marrow toxicity

<table>
<thead>
<tr>
<th>Patient</th>
<th>AZA dosage (mg/kg/day)</th>
<th>Days of AZA treatment*</th>
<th>Hemoglobin (mmoles/liter) Before AZA</th>
<th>WBC† (x 10⁹/liter)</th>
<th>Platelets (x 10⁹/liter)</th>
<th>Hemoglobin (mmoles/liter) At nadir</th>
<th>WBC (x 10⁹/liter)</th>
<th>Platelets (x 10⁹/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>30</td>
<td>6.7</td>
<td>6.0</td>
<td>303</td>
<td>4.5</td>
<td>1.4</td>
<td>102</td>
</tr>
<tr>
<td>2</td>
<td>1.5</td>
<td>28</td>
<td>7.4</td>
<td>3.9</td>
<td>188</td>
<td>4.9</td>
<td>1.4</td>
<td>4</td>
</tr>
<tr>
<td>3*</td>
<td>1.8</td>
<td>56</td>
<td>7.5</td>
<td>4.1</td>
<td>296</td>
<td>7.2</td>
<td>0.8</td>
<td>280</td>
</tr>
</tbody>
</table>

* Number of treatment days before bone marrow toxicity occurred.
† WBC = white blood cell count.
‡ See also ref. 6.

According to methods described previously (7,8). Briefly, for PNP and 5NT activities, after cell separation by a Percoll gradient, lymphocyte numbers were determined in a Coulter counter (Coulter Electronics, Luton, England), and exact numbers of cells were collected in Eppendorf tubes for each assay. The cell suspension was freeze-dried and enzyme assays were carried out by a previously described radiochemical micromethod (7). PNP was assayed in the anabolic direction, by which 1⁴C-labeled hypoxanthine is converted into 1⁴C-labeled inosine. Total 5NT was measured by the degradation of 1⁴C-labeled AMP to 1⁴C-labeled adenosine. For the TPMT assay an erythrocyte lysate was transferred (without the "ghost cells") to 1-ml Eppendorf tubes and frozen at -70°C until further use. TPMT activity was determined by measuring the methyl-transfer of S-adenosyl-L-[methyl-1⁴C]-methionine to 6-[methyl-1⁴C]-mercaptopurine (8,9). Activities of 5NT and PNP were expressed in nmoles/10⁹ lymphocytes/hour⁻¹, and TPMT in pmoles/10⁹ erythrocytes/hour⁻¹. All assays were performed in quadruplicate.

RESULTS

Blood samples for measurement of purine enzyme activities in the patients were obtained at least 3 months after hematologic abnormalities had resolved. None of the subjects studied had received blood transfusions within the 4 months prior to blood drawing for purine enzyme measurement. All subjects had normal kidney and liver function test results and

Table 2. TPMT and 5NT activities in 3 rheumatoid arthritis patients with azathioprine-related bone marrow toxicity and 16 control rheumatoid arthritis patients (controls)*

<table>
<thead>
<tr>
<th></th>
<th>Controls, median (range)</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TPMT</td>
<td>22.75 (12.24-41.11)</td>
<td>7.43</td>
<td>0</td>
<td>28.15</td>
</tr>
<tr>
<td>5NT</td>
<td>7.42 (3.72-19.18)</td>
<td>9.55</td>
<td>5.52</td>
<td>2.49†</td>
</tr>
</tbody>
</table>

* TPMT = thiopurine methyltransferase (pmoles/10⁶ erythrocytes/hour⁻¹); 5NT = 5'-nucleotidase (nmoles/10⁶ lymphocytes/hour⁻¹). † See also ref. 6.

biweekly, was recommenced. Over a followup period of 1½ years, no hematologic problems have reoccurred.

Patient 2, a 49-year-old woman who had RF-positive RA since 1974, has been described in more detail elsewhere (5). Previous treatment consisted of hydroxychloroquine, aurothioglucose, and D-penicillamine. The latter had to be discontinued because of mild leukopenia (2.4 x 10⁹/liter) and aurothioglucose, and D-penicillamine. The latter had to be

...
normal serum urate levels. The mean coefficient of variation within assays was 0.10, 0.07, and 0.03 for 5NT, PNP, and TPMT, respectively. Interassay variation (5 controls, 3 consecutive weeks) ranged between 0.10 and 0.19 for 5NT, between 0.05 and 0.23 for PNP, and between 0.04 and 0.07 for TPMT. PNP activity in patients experiencing AZA-related bone marrow toxicity was within the range of values found in the control group (data not shown). Patient 1 was found to have a complete TPMT deficiency, and TPMT activity was diminished in patient 2. 5NT activity was low in patient 3 when compared with the control subjects (Table 2).

DISCUSSION

All 3 patients with acute azathioprine-related bone marrow toxicity had a deficiency of 1 of the purine enzymes measured. Two of them had a TPMT deficiency, 1 complete and 1 partial. In the third patient, low activity of 5NT was found. The formation of cytotoxic thionucleotides and their feedback inhibition on de novo purine synthesis are considered to be responsible for both the therapeutic and the adverse effects of purine analogs. Impaired catabolism of thiopurines will lead to the accumulation of thionucleotides and enhance cytotoxicity (Figure 1); bone marrow depression during concurrent use of AZA and allopurinol, a xanthine oxidase antagonist, is an example of this (5,10). None of the patients described herein was taking allopurinol at the time bone marrow toxicity occurred, and the absence of decreased serum urate levels sufficiently excludes the possibility of xanthine oxidase deficiency as a cause of AZA toxicity.

TPMT is inherited in an autosomal codominant manner and shows a wide range of activity in the healthy population (9,11,12). Approximately 1 in 300 individuals has a complete deficiency of TPMT, and 11% show intermediate activity (8,9). Thus far, TPMT deficiency as a cause of AZA-related bone marrow depression has been reported in 8 patients (1-3), including 1 with RA (1). It was found to be associated with high levels of 6-thioguanine-nucleotide (1).

Our observations in patients 1 and 2 confirm the association of TPMT deficiency with AZA-related bone marrow toxicity. Activity of 5NT measured in patient 3 was well below the lowest value found in the RA patients who served as a control group. This condition may lead to increased levels of thionucleotides, causing increased susceptibility to bone marrow toxicity during treatment with AZA.

Two major forms of 5NT are recognized: ecto-5NT and cytosolic 5NT (13). Ecto-5NT is located on outer membranes, which are not permeable for thionucleotides, and most likely plays no role in the intracellular thiopurine metabolism and its subsequent cytotoxicity (14,15). This is supported by the observation that in vitro drug resistance to 6-thioguanine in childhood lymphoblastic leukemia is not related to ecto-5NT activity and is associated with high cytosolic 5NT activity (16). Our assay measured total 5NT activity. It seems reasonable that low cytosolic 5NT would account for the low total 5NT activity levels we recently found to be associated with AZA-related bone marrow toxicity in 3 patients, including the 1 reported here and 2 renal transplant recipients (6). 5NT activity varies between lymphocyte subsets and with maturation state. Although the use of a control group of patients with RA, all of whom had taken AZA, makes it less likely that such differences will have influenced our results, this possibility cannot be fully excluded.

In conclusion, deficiency of purine enzymes, including TPMT and 5NT, may be a cause of azathioprine-related bone marrow toxicity. If low activity of TPMT or 5NT is found in a patient who is believed to have AZA-related bone marrow toxicity, a rechallenge should be avoided, or, when inevitable, the drug should be started at a lower dosage and with close monitoring of hematologic parameters. In relatives of a person who has experienced AZA-related bone marrow toxicity or one who has been shown to have a TPMT deficiency, determination of TPMT activity before the start of AZA treatment is suggested. Although at present, measurement of purine enzymes is laborious, it is conceivable that when methods become less expensive and more readily available, this potentially life-threatening side effect of AZA can be prevented.

REFERENCES