Case Report

Azathioprine-induced pure red-cell aplasia

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Introduction

Long-term administration of azathioprine is widely used to prevent rejection in organ transplantation and in the treatment of autoimmune diseases. Bone marrow toxicity is a well-known side-effect of azathioprine. Macrocytosis, with or without megaloblastic erythropoiesis in the bone marrow, and (mild) leukopenia are frequently seen \cite{1-4}. Less common effects are anaemia, thrombocytopenia and pancytopenia \cite{5,6}. Pure red-cell aplasia is a rare complication of azathioprine, which was first reported in two patients by McGrath \textit{et al.} in 1975 \cite{1}. So far, no more than 10 cases have been reported in the literature \cite{1,2,7-12}. We describe this disorder in a renal transplant patient. Discontinuation of the drug resulted in full recovery.

Case report

A 48-year-old female with insulin-dependent diabetes mellitus received a cadaveric kidney transplant in 1984 because of end-stage kidney disease caused by diabetic nephropathy. Immunosuppressive therapy consisted of prednisone and cyclosporin. Five years after transplantation her renal function deteriorated, which was ascribed to cyclosporin nephrotoxicity. The medication was changed to prednisone and azathioprine. Her renal function improved (estimated creatinine clearance 30 ml/min). In 1994 she was admitted to our hospital because of progressive fatigue, dyspnoea, and pain in her left arm on exertion. Her urine production had remained normal. The diabetes mellitus was well regulated. There was no history of fever, chills, or faecal blood loss. Her medication consisted of prednisone (10 mg daily), azathioprine (50 mg daily), folic acid, insulin, acetylsalicylic acid, and norfloxacin.

On physical examination the patient looked pale. The blood pressure was 150/70 mmHg. Except for a systolic heart murmur due to mitral valve insufficiency, no other abnormalities were found. The laboratory examination yielded the following results: haemoglobin: 4.7 mmol/l (normal: 7.5–10.0 mmol/l); haematocrit: 0.22 (normal: 0.37–0.47); mean corpuscular volume (MCV): 103 fL (normal: 80–100 fL); reticulocyte count: <0.002 (normal: 0.002–0.020). The leucocyte count, differentiation, and the thrombocyte count were normal. Ferritin, folic acid, and vitamin B\textsubscript{12} were within the normal range. The creatinine clearance was 25 ml/min. The liver enzymes were not elevated, and the direct Coombs test was negative. No autoantibodies (antinuclear factor, anti-DNA antibodies, and rheumatoid factor) were detected. A stool examination for microscopic blood loss was negative. Chest X-ray was normal. The serological tests for cytomegalovirus, Epstein–Barr virus, hepatitis B virus, and parvovirus B19 were negative.

Since there were no signs of either blood loss or haemolysis and the patient's renal graft function had not deteriorated markedly, a bone marrow examination was done. The bone marrow cytology showed erythroid hypoplasia with an erythroid:myeloid ratio of 1:200. The myelopoesis and megalakaryopoesis were normal as was the presence of iron. These results were compatible with pure red-cell aplasia. Since a recent virus infection or any other causes for pure red-cell aplasia could not be found, drug-induced pure red-cell aplasia was considered. As azathioprine appeared to be the most likely causative drug, it was discontinued and immunosuppressive therapy was changed to prednisone and cyclosporin. Because of angina pectoris, the patient received four units of packed red cells. After the discontinuation of azathioprine, the reticulocyte fraction increased to 0.52 after 12 weeks. Her haemoglobin climbed to 9.6 mmol/l without further blood transfusions. The renal function remained stable. Since cytotoxicity due to azathioprine has been associated with enzyme deficiencies of thiopurine methyltransferase (TPMT) or 5'-nucleotidase (5'-NT), we measured these enzyme activities in our patient's leucocytes (TPMT 24.55 ± 1.25 nmol/h/10\textsuperscript{6} cells (normal range: 16.5–25.6, mean ± SD); 5'-NT 10.93 ± 0.79 nmol/h/10\textsuperscript{6} cells (normal range: 12.2–27.8, mean ± SD)).
Discussion

Pure red-cell aplasia is a specific syndrome characterized by anaemia, reticulocytopenia, and strikingly reduced or absent erythroid precursor cells in the bone marrow. Pure red-cell aplasia is an inherited or primary or secondary acquired disorder. Secondary acquired forms can be observed after (viral) infections (especially human parvovirus B19), autoimmune diseases, thymoma, haematological malignancies, chronic haemolytic anaemias, severe nutritional deficiencies, and (certain) drugs and toxins [13].

Pure red-cell aplasia is a rare complication of azathioprine that was first reported in two patients by McGrath et al. in 1975 [1]. Since then only eight other cases of azathioprine-induced pure red-cell aplasia have been reported (Table 1).

The underlying mechanism of drug-induced erythroid hypoplasia is poorly understood. In some cases immune-complex damage of erythroid progenitor cells or antibodies directed to antigens on these cells (modified by the drug) could be demonstrated [14]. In another case the effect was thought to be a direct, toxic drug inhibition of DNA synthesis of marrow erythroid cells [15]. Others failed to demonstrate these phenomena [11,16,17]. This could be due to the presence of alternate pathogenic mechanisms such as direct cellular toxicity or metabolite-mediated inhibition of erythropoiesis. Autoantibodies directed to erythropoietin seem not to be involved, because most patients have high erythropoietin titres in the blood when measured [1,11]. Furthermore, individually inherited variations in activity of thiopurine methyltransferase (TPMT) may be another factor in azathioprine toxicity.

After oral administration, azathioprine is rapidly converted to 6-mercaptopurine, which in turn undergoes extensive metabolism. In-vivo methylation is a major catabolic pathway catalysed by the enzyme TPMT. This enzyme is inherited in an autosomal co-dominant fashion and shows a wide range of activity in a healthy population. Very low TPMT activity (0.3% of the population) results usually in severe leukopenia or pancytopenia, appearing shortly after administration of azathioprine. Intermediate activity (11% of the population) renders patients more sensitive to azathioprine-induced myelosuppression [18,19]. So far pure red-cell aplasia has not been reported in these patients, but intermediate levels of TPMT activity could play a role in azathioprine-induced pure red-cell aplasia. Recently very low 5'-nucleotidase (5'-NT) activity in three patients with a history of azathioprine-related bone marrow suppression has also been reported (one case of pancytopenia and two with only leukopenia) [20]. Because of these reports, we measured both TPMT and 5'-NT in the serum of our patient. The level of TPMT was normal, whereas a borderline low level of 5'-NT was found. Although speculative, this could have played a role in the pathogenesis of the pure red-cell aplasia.

Azathioprine-induced pure red-cell aplasia is a rare complication and has so far been reported only in renal transplant recipients. Surprisingly, only one other case of poorly documented pure red-cell aplasia was reported in a patient with Crohn’s disease [10] and none in other organ transplantations or autoimmune diseases. All reported patients show a similar pattern, with a relatively long interval between initiation of treatment and onset of pure red-cell aplasia (2–7 years). Full recovery occurs within weeks of withdrawal of the azathioprine (Table 1). There were no laboratory parameters predicting development of pure red-cell aplasia. McGrath et al. advocated monitoring the mean red cell corpuscular volume (MCV) as a possible indicator [1]. However, Old et al. reported two patients with a normal MCV [7]. Moreover, macrocytosis is frequently seen without any myelosuppression during azathioprine treatment [1–4]. Measuring reticulocyte counts on a regular basis may help in early detection of pure red-cell aplasia.

After diagnosing pure red-cell aplasia, azathioprine should be discontinued. Complete withdrawal of azathioprine is mandatory for full erythroid recovery. Merely reducing the dose does not reverse the pure

<table>
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<tr>
<th>Reference</th>
<th>Number of patients</th>
<th>Age (years)</th>
<th>MCV*</th>
<th>Disease</th>
<th>Interval* (years)</th>
<th>Time until Recovery (weeks)</th>
<th>Azathioprine dose (daily)</th>
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<td>117</td>
<td>Kidney transplant</td>
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<td>90</td>
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<td>12</td>
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<td>—</td>
<td>—</td>
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<td>48</td>
<td>103</td>
<td>Kidney transplant</td>
<td>5</td>
<td>12</td>
<td>1 mg kg</td>
</tr>
</tbody>
</table>

*Mean corpuscular volume; *Normal; *Interval between initiation of azathioprine and occurrence of pure red-cell aplasia.
Azathioprine-induced pure red-cell aplasia

red-cell aplasia [9]. Re-administering the drug at a lower dose following full erythroid recovery sometimes seems possible without recurrence of the anaemia [1,2]. In most cases, however, alternative immunosuppressive therapy has to be used.

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References

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