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Severe megaloblastic anaemia associated with abnormal azathioprine metabolism

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Severe anaemia is a rare, unexplained, side effect of azathioprine therapy. We report here such a case associated with a previously unreported abnormality in azathioprine metabolism. A 57 year old man on azathioprine therapy, for a presumed collagen vascular disease, developed severe megaloblastic anaemia. This resolved on cessation of azathioprine treatment. A very high concentration of an azathioprine metabolite, 6-thioguanine nucleotide, was found in the patient's red blood cells and this was confirmed by subsequent rechallenge with a single dose of 50 mg azathioprine.

Keywords azathioprine anaemia metabolism

Introduction

Macrocytosis and megaloblastic changes in the bone marrow are commonly seen after treatment with azathioprine (Wickramasinghe *et al.*, 1974). However, severe anaemia is a rare side-effect of azathioprine, although some cases of selective erythroid hypoplasia have been reported. We report here a patient who developed severe anaemia whilst taking azathioprine and whose bone marrow showed megaloblastic erythropoiesis.

We considered that his anaemia was either due to an abnormally sensitive bone marrow, possibly influenced by previous cyclophosphamide therapy, or to exposure to high concentrations of active azathioprine metabolite. To test the latter hypothesis we assayed red blood cell (RBC) 6-thioguanine nucleotide (Lennard & Maddocks, 1983), an active metabolite of azathioprine.

Methods

A 57 year old man developed ulcers and livedo reticularis on his ankles 4 years ago. Treatment with cyclophosphamide for 18 months until February 1981 was ineffective. At this time his peripheral blood count was normal apart from lymphopenia.

Cyclophosphamide was stopped and azathioprine 100 mg daily and prednisone 10 mg daily prescribed. His leg ulcers healed and he was well until April 1981 when he complained of severe angina of effort. On examination he was pale and his blood count showed a haemoglobin of 6.8 g/100 ml, mean corpuscular volume 89 fl, white blood cell (WBC) count 2.7×10^9/l (neutrophils 84%, lymphocytes 10%, monocytes 5%, eosinophils 1%) platelets 308×10^9/l, and reticulocytes 0.4%. The blood film showed red cell anisocytosis, poikilocytosis and a few macrocytes. It was thought that his severe anaemia was possibly related to azathioprine therapy which was therefore stopped. Four days later bone marrow examination showed severe megaloblastic erythropoiesis. Granulopoiesis and lymphopoiesis were reduced but megakaryocytes were normal. Serum vitamin B_{12} was 260 ng/l, serum folate 18 μg/l, and red cell folate 257 μg/l. Treatment with prednisone was continued and after a blood transfusion, raising his haemoglobin to 12 g/100 ml, his angina ceased. Thirteen days after stopping azathioprine therapy he developed a reticulocytosis and his WBC count returned to normal. A repeat bone marrow aspirate showed normoblastic erythropoiesis.

A blood sample was obtained 26 days after stopping azathioprine. For comparison we used

the metabolite levels in kidney transplant recipients ($n = 30$), with stable functioning cadaver grafts and normal blood count, taking the same dose of azathioprine and prednisone.

In January 1982 his leg ulceration returned and the patient was challenged with a single 50 mg dose of azathioprine and blood samples taken at 0, 2, 4, 11, 20, 24, 44 h and 7 days. For comparison four patients with pemphigus vulgaris were used, one taking azathioprine alone and three taking azathioprine plus prednisolone (40 mg day^{-1}). Blood samples were obtained daily, 10 h post-dose, from the start of azathioprine therapy (50 mg twice daily). All samples were assayed for RBC 6-thioguanine nucleotide.

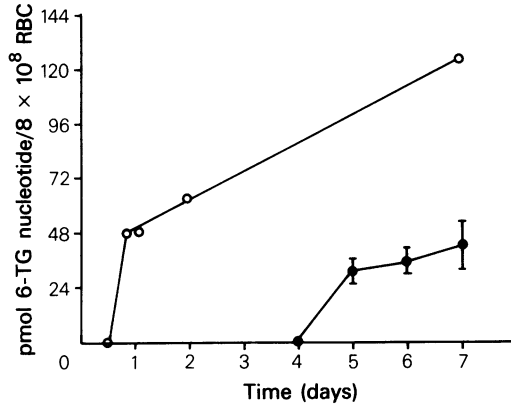

Figure 1 Red blood cell (RBC) 6-thioguanine (6-TG) nucleotide in the patient on rechallenge with a single oral dose of 50 mg azathioprine (○), compared to the mean (± s.e. mean) metabolite concentration in four patients with pemphigus vulgaris taking 50 mg twice daily azathioprine (●).

Results

The patient's 6-thioguanine nucleotide concentration, 26 days post-dose, was 365 pmol/8 × 10^8RBCs. The renal transplant recipients' mean 6-thioguanine nucleotide concentration, 10 h post-dose, was 140 (s.d. 71) pmol/8 × 10^8 RBCs, less than half the concentration measured in the patient.

On rechallenge with azathioprine the metabolite was detected in the patient's RBCs at 20 h, compared to 5 days in the pemphigus group, by 7 days the patient had nearly treble the concentration of RBC 6-thioguanine nucleotide (Figure 1).

Discussion

The patient's serum vitamin B_{12}, serum folate and red cell folate concentrations were normal. His megaloblastic anaemia responded to stopping azathioprine therapy indicating that it was probably drug induced.

There is strong evidence that the nucleotide metabolites of azathioprine are responsible for bone marrow toxicity. Children with the Lesch-Nyhan syndrome, unable to form nucleotide metabolites of the drug, do not develop bone marrow toxicity (Nyhan *et al.*, 1968) and we have shown a significant correlation between RBC 6-thioguanine nucleotide concentration and neutropenia in childhood lymphoblastic leukaemia (Lennard *et al.*, 1983) treated with 6-mercaptopurine. 6-Thioguanine nucleotide is a metabolite of both azathioprine and 6-mercaptopurine.

After the initial course of azathioprine this patient's RBC 6-thioguanine nucleotide concentration was much greater than that measured in haematologically normal renal transplant recipients. Rechallenging the patient with azathioprine produced metabolite concentrations far higher than those in the pemphigus group used for comparison. It was concluded that abnormal azathioprine metabolism was related to the cause of the severe megaloblastic anaemia. The precise mechanism is uncertain.

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