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Metabolism of azathioprine to 6-thioguanine nucleotides in patients with pemphigus vulgaris

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Azathioprine metabolism, to red cell 6-thioguanine nucleotides (6TGN), was studied in four patients with pemphigus vulgaris. Throughout treatment blood samples were taken for red cell 6TGN assay and differential white cell counts. Metabolite steady-state occurred in three patients in 2, 2 and 4 months respectively. In the fourth patient red cell 6TGN concentrations increased slowly over 3 years. There was a significant negative correlation between 6TGN concentrations and the white blood cell count ($r_s = -0.92$, $P < 0.0005$) in this patient.

Keywords azathioprine cytotoxic metabolism thiopurines

Introduction

Pemphigus vulgaris is an autoimmune disease mediated by antibodies to surface antigens on keratinocytes (Ahmed *et al.*, 1984). Systemic corticosteroids are used for the treatment of the disease but, because of the severe side effects associated with the prolonged use of these drugs, azathioprine is frequently used for adjuvant therapy. In patients with less severe disease concurrent azathioprine has been shown to reduce the maintenance dose of steroid (Lever & Schaumburg-Lever, 1977, 1984). However, long term immunosuppressive therapy is associated with serious side effects; including bone marrow toxicity and an increased risk of cancer (Penn, 1978; Kinlein *et al.*, 1979).

Azathioprine is rapidly cleaved to form 6-mercaptopurine *in vivo*. The cytotoxic activity of 6-mercaptopurine is due, in part, to the incorporation of 6-mercaptopurine derived 6-thioguanine nucleotides into DNA (Tidd & Paterson, 1974). 6-Thioguanine nucleotides (6TGN) are the major thiopurine metabolites in the human red blood cell (RBC) (Lennard & Maddocks, 1983). We have shown large individual variations in RBC 6TGN concentrations among patients on identical doses of azathioprine or 6-mercaptopurine (Lennard *et al.*, 1983, 1984a). Raised concentrations of RBC 6TGN have been found in patients with bone marrow depression due to azathioprine and 6-mercaptopurine (Lennard *et al.*, 1984a,b). We have also shown that raised RBC 6TGN concentrations are associated with actinic keratoses and malignant skin tumours in renal transplant recipients on long term azathioprine therapy (Lennard *et al.*, 1985).

Azathioprine metabolism was studied in four patients with pemphigus vulgaris with the aim of determining the time required to reach steady-state concentrations of 6TGN. Long term intra-patient variations and relationships with the white blood cell count were also investigated.

Methods

Study design

Four patients (two men, two women), aged 38 to 52 years, with pemphigus vulgaris were studied, three from initial diagnosis and one 5 months after diagnosis. All the patients had normal liver

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and renal function. Clinical diagnosis was confirmed by direct immunofluorescence of a skin biopsy showing positive staining of intercellular substance (Beutner & Nisengard, 1973). At the start of treatment, and at each clinic visit thereafter, the skin and mucosal lesions were visually graded: 0 = absent, 1 = mild, 2 = moderate and 3 = severe activity. Throughout treatment blood samples were taken for RBC 6TGN assay and differential blood cell counts, and skin biopsies were performed for direct immunofluorescence measurements. Lesion grading was performed blind to the results of 6TGN assay.

Drug protocol

Patients with mild pemphigus were treated with daily oral prednisolone and azathioprine. Treatment was individualized for each patient depending on the severity of the disease. The starting doses of prednisolone (30 to 40 mg, once daily) and azathioprine (50 mg twice daily) were constant until the rate of new lesion production decreased and the bulk of those lesions healed. The steroid dose was then gradually reduced, by 2.5 or 5 mg weekly, to the lowest level that suppressed new lesion formation. The azathioprine dose was constant throughout the period of steroid dose reduction. It was increased by 50 mg daily on the appearance of new lesions or if the original lesions proved difficult to control. It was decreased in response to a rapidly falling white blood cell count or a prolonged period of lesion absence.

6TGN assay

Blood samples were obtained at least 5 h post-dose in a lithium heparin tube. The washed counted RBCs were stored at −20° C until assayed. RBC 6TGN concentrations were measured by fluorimetric assay (Lennard & Maddocks, 1983). The lower limit of sensitivity for the assay was 30 pmol/8 × 10^8 RBCs and the coefficient of variation (c.v.), for 30 assays over a 3 year period, was 10.2% at 120 pmol/8 × 10^8 RBCs and 8.5% at 600 pmol/8 × 10^8 RBCs.

Results

Skin lesions cleared after 2.5 to 5 (mean 3.6) weeks drug treatment. Mouth lesions initially cleared after 3 to 12.8 (mean 6.1) months treatment. Table 1 shows lesion scoring, azathioprine dose adjustments and RBC 6TGN concentrations, for each patient, at every clinic visit at which lesion grading changed, the azathioprine dose was adjusted or a skin biopsy performed.

Patient 1 (man, 16 assays over 39 months) achieved steady state 6TGN concentrations after 2 months. From 2 to 39 months the mean 6TGN concentration was 234 pmol/8 × 10^8 RBCs (c.v. 5.2%, $n = 9$). The azathioprine dosage was not constant throughout therapy. It was increased from 100 to 150 mg daily at 1 month and decreased from 150 to 100 mg daily at 15 months. The latter decrease had no observable effect on RBC 6TGN steady state concentrations which had a c.v. of 3.7% ($n = 5$) over the period 15 to 39 months. There was no relationship between RBC 6TGN concentrations and the white blood cell count ($r_s = -0.28$, $n = 16$, $P > 0.1$).

Patient 2 (man, 18 assays over 18 months) reached steady state 6TGN concentrations after 4 months azathioprine therapy. From 4 to 18 months of treatment the mean 6TGN concentration was 177 pmol/8 × 10^8 RBCs (c.v. 10.1%, $n = 11$). The azathioprine dose was increased from 100 to 150 mg daily after 12 months but RBC 6TGN measurements up to 18 months remained constant (c.v. 4 to 12 months = 8.8%, $n = 7$; c.v. 13 to 18 months = 10.6%, $n = 4$). There was no relationship between RBC 6TGN concentrations and the white blood cell count ($r_s = -0.36$, $n = 18$, $P > 0.5$).

Patient 3 (woman, 21 assays over 36 months) was studied daily for the first week of azathioprine therapy. RBC 6TGN was detected from day 7 and reached steady state at 2 months. From 2 to 36 months the mean 6TGN concentration was 122 pmol/8 × 10^8 RBCs (c.v. 8.2%, $n = 10$). The azathioprine dose, 100 mg daily, was constant throughout. There was no relationship between RBC 6TGN concentrations and the white blood cell count ($r_s = -0.24$, $n = 21$, $P > 0.1$.

Patient 4 (woman, 14 assays over 40 months) did not achieve 6TGN steady state. 6TGN concentrations increased steadily over 3 years until, in response to a falling white cell count, dose reduction occurred. There was a statistically significant relationship between RBC 6TGN concentrations and the white cell count ($r_s = -0.92$, $n = 10$, $P < 0.0005$; Figure 1).

Discussion

These results show a slow accumulation of 6TGN in the RBC. Three patients attained steady-state levels after several weeks azathioprine therapy. One patient did not achieve steady-state and a statistically significant relationship between

Table 1 Pemphigus vulgaris. Disease activity, drug doses and metabolite concentrations at intervals throughout treatment. The direct immunofluorescence (I.F.), staining of skin biopsies was scored + = positive, − = negative. Skin (S) and Mouth (M) lesions were scored 0 = absent, 1 = mild, 2 = moderate, 3 = severe.

Patient	*Time (months)*	*Direct I.F.*	*Lesion grade* S	M	*Prednisolone (mg)*	*Azathioprine (mg)*	*6TGN (pmol/ 8×10^8 RBCs)*
1	0	+	1	1	30	100	0
	1		1	1	30	150	102
	1.2		0	1	30	150	120
	6		0	1	20	150	234
	12.8		0	0	12.5	150	240
	15		0	0	12.5	100	228
	24		0	0	12.5	100	240
	36	+	0	0	7.5	100	240
2	0	+	1	3	40	100	0
	0.6		0	2	30	100	78
	1		0	1	30	100	84
	4.5		0	0	10	100	180
	6	−	0	1	7.5	100	192
	12	+	0	2	10	150	198
	18		0	1	10	150	210
3	0	+	2	0	40	100	0
	0.5		1	0	20	100	72
	1	+	0	0	17.5	100	84
	6	+	0	0	10	100	114
	12	+	0	0	7.5	100	96
	24	−	0	0	5	100	138
	36	−	0	0	2.5	100	126
4	0	+	1	1	40	150	−
	0.7		0	1	30	150	−
	1		0	1	20	150	−
	3.0		0	0	12.5	150	−
	6	+	0	0	10.0	150	48
	12		0	0	8	150	72
	24		0	0	8	150	114
	33		0	0	6	150	174
	36	+	0	0	6	150	371
	38		0	0	6	100	420
	40	−	0	0	6	100	323

increasing 6TGN concentrations and falling white cell count was observed. The changing weight of this patient may have influenced azathioprine metabolism. The patient's weight increased slowly, from 82 kg at diagnosis, to 87 kg at 2.6 years. A change in diet at 2.6 years resulted in a weight loss of 11 kg by 3.17 years. This patient had no active disease during this period, accordingly her prednisolone dose was reduced from 8 to 6 mg daily at 2.75 years. In this individual there was a decrease in RBC 6TGN concentrations on azathioprine dose reduction. However, in two other patients, dose adjustments were not reflected by changes in 6TGN concentrations.

The precise mode of action of azathioprine as an immunosuppressive drug is uncertain. The immunosuppressive activity of azathioprine could be explained by the cytotoxic and inhibitory effects of 6-mercaptopurine on cellular proliferation. However, evidence has been presented for additional mechanisms of action for azathioprine in the suppression of immune responses (Bach & Dardenne, 1972; Al-Safi & Maddocks, 1983, 1984; Szawlowski *et al.*, 1985). We have no measure of immunosuppression in these patients. The direct immunofluorescence of skin biopsy specimens, showing positive staining of anti-epidermal antibody, is a qualitative measure of disease activity.

Some individuals on chronic azathioprine therapy (e.g. patient 4), for unknown reasons, continue to accumulate 6TGN in their RBCs and

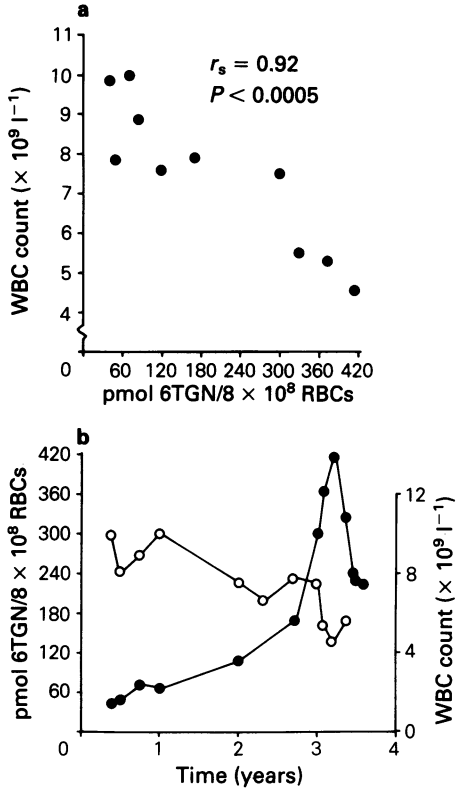

Figure 1 The relationship between 6TGN concentration and white blood cell (WBC) count (a), and 6TGN accumulation with time (b). • = 6TGN concentration; ○ = WBC count) in Patient 4. The azathioprine dose was reduced from 150 mg to 100 mg daily at 3.17 years.

develop a gradual and progressive leucopenia. This supports the need for continued monitoring for toxic effects throughout therapy, even for those patients on constant dose, long-term treatment (ABPI, 1985).

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