CONCISE REPORTS

6-Thioguanine nucleotide accumulation in erythrocytes during azathioprine treatment for systemic connective tissue diseases: a possible index for monitoring treatment

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Abstract

Background Owing to adverse pharmacokinetics, azathioprine treatment may fail to induce a satisfactory clinical response in systemic connective tissue diseases. The major intracellular cytotoxic metabolites of azathioprine are 6-thioguanine nucleotides (6TGNs).

Methods To assess whether the erythrocyte accumulation of 6TGN is a clinically applicable index for monitoring azathioprine treatment, erythrocyte accumulation of 6TGN was measured in patients with rheumatoid arthritis (n=12), systemic lupus erythematosus (n=7), polyarteritis nodosa (n=2), myositis (n=1), or leucocytoclastic vasculitis (n=1). Ages ranged from 28 to 75 (median 58) years.

Results Erythrocyte accumulation of 6TGN varied among the patients from 20 to 303 (median value 95) nmol/mmol haemoglobin. No significant correlation was found between erythrocyte accumulation of 6TGN and the dose of azathioprine/kg body weight, the age of the patients, the duration of treatment, or the presence of myelotoxicity or hepatotoxicity. The interindividual coefficient of variation (CV) in the erythrocyte accumulation of 6TGN/mg azathioprine/kg body weight was 0.65. The median intraindividual CV in erythrocyte accumulation of 6TGN at an unchanged dose of azathioprine was 0.09 (19 patients; range 0.03-0.27).

Conclusions The low intraindividual variation compared with the high interpatient variation in erythrocyte accumulation of 6TGN implies that erythrocyte accumulation of 6TGN may be clinically applicable for monitoring azathioprine treatment. Prospective studies are needed to clarify the relation between the erythrocyte accumulation of 6TGN and the clinical response to treatment, and to establish recommendations for dose modifications.

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For more than 20 years the thiopurine analogue azathioprine has been used to treat systemic connective tissue diseases.¹⁻³ The response to azathioprine treatment is unpredictable, however, and often disappointing and the optimal

regimen for individual dose adjustments has still to be established.

The thiopurines are prodrugs with considerable and clinically relevant interindividual variations in their metabolism.⁴⁻⁵ After absorption, azathioprine is cleaved to 6-mercaptopurine, which, after several metabolic steps, is incorporated into DNA and RNA as 6-thioguanine nucleotides (6TGNs), thereby mediating the cytotoxic and immunosuppressive effects of azathioprine.⁴⁻⁶ During thiopurine treatment, 6TGN accumulates in cells, including erythrocytes.⁷ As erythrocyte accumulation of 6TGN may reflect systemic exposure to thiopurine as well as the individual patient's ability to accumulate 6TGN, erythrocyte accumulation of 6TGN could be an index of intensity of treatment.⁷⁻⁹ As part of a continuing study to evaluate the possible usefulness of erythrocyte accumulation of 6TGN as a guide for adjustments of dose of azathioprine in patients with systemic connective tissue diseases, we report on the inter- and intraindividual variations in erythrocyte accumulation of 6TGN in relation to the characteristics of patients and to toxicity.

Patients and methods

PATIENTS

We studied 23 patients with systemic connective tissue diseases who were treated with azathioprine at the medical department TTA, University Hospital, Copenhagen. The table gives the data on patients. All had normal kidney function as measured by serum creatinine.

TREATMENT

The table and the figure give the characteristics of azathioprine treatment. For all patients the dose of azathioprine was unchanged during the study period. As well as azathioprine, 13 patients received prednisone throughout the study and six patients received non-steroidal anti-inflammatory drugs. Otherwise the patients received no drugs known to affect disease activity or metabolism of azathioprine.

RESPONSE TO TREATMENT

As the design of the study was retrospective and the clinical response to azathioprine was not uniformly registered, it was not possible to assess reliably the response to azathioprine treatment. The correlations between erythrocyte accumulation of 6TGN and the following

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Characteristics of patients

Diagnosis	Sex	Age (years)	Duration of SCTD (years)	Starting dose PRED (mg/kg)	Starting dose AZA (mg/kg)	Years of AZA treatment	At study period	
							Dose PRED (mg/kg)	Mean E-6TGN†/mg AZA/kg
RA	M	41	25	0.20	1.3	2	0.10	89
RA	M	54	33		1.4	5		107
RA	W	57	42	0.28	1.4	7	0.14	14
RA	W	65	7		1.3	4		32
RA	W	65	36		1.6	7		51
RA	W	66	39		1.8	8		32
RA	W	66	9	0.08	2.5	2	0.00	81
RA	M	67	13	0.15	1.5	7	0.15	48
RA	M	69	35	0.35	2.5	17	0.09	233
RA	W	69	11		2.6	5		48
RA	W	73	19	0.45	1.8	2	0.14	40
RA	W	75	21		3.2	8		71
SLE	W	29	9	0.56	2.1	9	0.11	61
SLE	W	37	24	0.25	2.5	4	0.21	26
SLE	W	41	13	0.72	2.4	1	0.24	53
SLE	W	43	20	0.32	2.3	16	0.08	86
SLE	W	43	15	0.51	2.6	2	0.08	60
SLE	W	47	17	0.20	1.4	4	0.00	112
SLE	W	48	9	1.20	1.7	4	0.18	91
PAN	W	28	5	1.67	2.5	3	0.33	38
PAN	M	63	18	0.98	1.5	4	0.00	151
Leu vas	W	42	8	0.64	0.8	3	0.00	46
MYO	W	59	29	0.18	1.8	9	0.00	80

RA=Rheumatoid arthritis; E-6TGN=erythrocyte accumulation of 6TGN; SLE=systemic lupus erythematosus; PAN=polyarteritis nodosa; Leu vas=leucocytoclastic vasculitis; MYO=myositis; W=woman; M=man; SCTD=systemic connective tissue disease; PRED=prednisone; AZA=azathioprine.
*Dose of steroids and azathioprine when azathioprine treatment was initiated.
†Mean erythrocyte accumulation of 6-thioguanine nucleotides at an unchanged dose of azathioprine (nmol/mmol haemoglobin).

indices were calculated: haemoglobin, white cell count, absolute neutrophil count, lymphocyte count, thrombocyte count, erythrocyte sedimentation rate, C reactive protein concentrations, level of antibodies to DNA, and serum aminotransferase activity. For each patient a mean of all measurements available during the study period for each of these variables was calculated.

Erythrocyte accumulation of 6TGN was measured by high performance liquid chromatography as described elsewhere.¹⁰ The within and between run coefficients of variation (CVs) of the assay were between 5 and 7%. Erythrocyte accumulation of 6TGN was always assayed in duplicate and expressed as nmol 6TGN/mmol haemoglobin. At an unchanged dose of azathio-

prine, erythrocyte accumulation of 6TGN was measured once in four patients, twice in three patients, three times in 14 patients, and four times in two patients. A mean of these measurements was calculated for each patient.

STATISTICAL ANALYSIS

Subgroups were compared for differences in specified variables with the Mann-Whitney rank sum test. The correlation between variables was tested with Spearman's non-parametric rank correlation analysis (r_s =Spearman's correlation coefficient). Values of $p<0.05$ were regarded as statistically significant.

Results

Mean erythrocyte accumulation of 6TGN varied among the patients from 20 to 303 nmol/mmol haemoglobin (median value 95; figure). The erythrocyte accumulation of 6TGN in relation to the prescribed dose of azathioprine (mean erythrocyte accumulation of 6TGN/mg azathioprine/kg body weight) ranged from 14 to 233 nmol/mmol haemoglobin/mg azathioprine/kg (median value 60) with an inter-individual CV of 0.65. The erythrocyte accumulation of 6TGN/mg azathioprine/kg was not significantly related to duration of disease, age, concurrent administration of prednisone, the total preceding period of azathioprine treatment, or the haemoglobin concentration. Mean erythrocyte accumulation of 6TGN was not significantly related to the dose of azathioprine/kg ($p=0.36$; figure). Men had a higher erythrocyte accumulation of 6TGN/mg azathioprine/kg than women ($p=0.01$; table). The median intraindividual CV of erythrocyte accumulation of 6TGN at an unchanged dose of azathioprine was 0.09 (19 patients; range 0.03–0.27), and this was not significantly related to erythrocyte accumulation of 6TGN, haemoglobin, the dose of azathioprine, the number of

Scattergram of steady state levels of the erythrocyte accumulation of 6-thioguanine nucleotides (E-6TGNs) in relation to the daily oral dose of azathioprine/kg body weight.

measurements of erythrocyte accumulation of 6TGN, or sex.

The prevalence of myelotoxicity was low. When all patients were included, the median platelet count was $288 \times 10^9/l$, the median white blood cell count $6.4 \times 10^9/l$, the median absolute neutrophil count $4.7 \times 10^9/l$, and the median lymphocyte count $1.0 \times 10^9/l$. No significant correlations between erythrocyte accumulation of 6TGN and the haematological variables were found for the total study group or for disease subgroups. No patients had serum aminotransferase activity above the upper normal limit (40 IU/l). No correlations were found between mean erythrocyte accumulation of 6TGN and the sedimentation rate or C reactive protein concentration. All three patients with systemic lupus erythematosus and anti-DNA levels >10 IU/l had mean erythrocyte accumulation of 6TGN of <90 nmol/mmol haemoglobin.

Discussion

For several decades azathioprine treatment has been used to induce remissions or reduce the need for prednisone in patients with systemic connective tissue diseases. A major reason for failures of azathioprine treatment might be individual variations in thiopurine pharmacokinetics reflecting a complex integration of genetically and behaviourally determined factors.^{11, 12} Owing to the small intraindividual and large interindividual variations in erythrocyte accumulation of 6TGN found in the present study, ranking according to the amount of erythrocyte accumulation of 6TGN with a single or a few measurements of erythrocyte accumulation of 6TGN is possible. Most of the patients in this study had far lower erythrocyte accumulation of 6TGN values than those necessary for 6-mercaptopurine to induce optimal treatment results in acute lymphoblastic leukaemia in childhood.¹² Although it remains to be determined whether failures of azathioprine treatment in patients with systemic connective tissue diseases are similarly due to adverse drug metabolism and are reflected in low erythrocyte accumulation of 6TGN, the interindividual differences in pharmacokinetics do imply variations in treatment intensity at similar drug dosage. Hence indices are needed that reflect these differences and that may serve as guidelines for adjustments of dose.

Because lymphocytes lack xanthine oxidase, a major enzyme in thiopurine metabolism with little interindividual variation in activity,¹³ concentrations of 6TGN may be higher in

erythrocytes than in lymphocytes; however, as in vivo data on leucocyte-6TGN are lacking the clinical significance of this is unknown.

As none of the patients showed clinical myelo- or hepatotoxicity, increases in dose would have been possible in most cases. Treating to toxicity may not always be necessary, however, as several of the patients with the highest erythrocyte accumulation of 6TGN in the present study had a satisfactory clinical response and still only moderate or no signs of toxicity. Until the applicability and clinical value of erythrocyte accumulation of 6TGN as a guideline for adjustments of that dose of azathioprine have been prospectively evaluated, we recommend that the dose of azathioprine be increased for patients not responding to standard doses and in whom dose limiting hepato- or myelotoxicity has not developed.

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