

CONCISE REPORTS

Purine enzymes in rheumatoid arthritis: possible association with response to azathioprine. A pilot study

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Abstract

Objective—To study the possible association of purine enzyme activities with response to azathioprine (AZA) treatment in rheumatoid arthritis (RA) and their correlation with parameters of disease activity.

Patients and methods—Lymphocyte activities of hypoxanthine-guanine phosphoribosyl-transferase (HGPRT), adenine phosphoribosyltransferase (APRT), purine nucleoside phosphorylase (PNP) and 5'-nucleotidase (5NT), and erythrocyte activities of thiopurine methyltransferase (TPMT) were measured in 14 healthy controls and 36 patients with RA. Eight patients had not previously been treated with AZA. Response to AZA therapy in 28 patients, determined in a prospective trial, was considered good in nine (group 1), insufficient in seven (group 2). In 12 patients AZA was withdrawn because of adverse reactions (group 3). Disease activity parameters were obtained simultaneously with purine enzyme measurements. Purine enzyme levels in the different groups were compared.

Results—Levels of 5NT activity were significantly lower in patients with RA than in healthy controls. PNP activity was higher in patients with RA not using prednisone compared with those who did and healthy controls. No clear correlation between purine enzyme levels and disease activity parameters was found. 5NT activities were significantly higher in group one than in group three ($p = 0.012$; $\alpha = 0.017$), and almost significantly higher than in group two ($p = 0.03$; $\alpha = 0.017$).

Conclusions—The results indicate that purine enzyme activities in patients with RA differ from healthy controls, are associated with the outcome of AZA treatment and seem not to be associated with disease activity. Our findings may offer a clue to predict the response to AZA therapy in RA.

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After conversion to 6-mercaptopurine (6MP) the metabolism of azathioprine (AZA) parallels the endogenous purine pathways. Deficiencies

of purine enzymes may reduce efficacy or increase toxicity of AZA.¹⁻³ To our knowledge, the relation between purine enzyme levels and response to AZA therapy in patients with RA has not been systematically studied so far. The present study was undertaken to investigate purine enzyme activities in patients with RA and their association with response to AZA therapy.

Patients and methods

PATIENTS

Thirty six patients with classic or definite RA⁴ were studied. Eight patients had not previously been exposed to AZA. The other 28 patients had been treated with AZA during a double-blind prospective trial of AZA and methotrexate,⁵ and could be assigned to three groups according to their response to AZA therapy. Group 1: patients with good clinical improvement within six months of treatment with AZA (100-150 mg daily), defined as >50% improvement of at least three of the following parameters: 1) patient's assessment of pain (visual analogue scale (VAS)); 2) Ritchie articular index (RAI)⁶; 3) erythrocyte sedimentation rate (ESR, Westergren); 4) duration of morning stiffness. Patients in group 2 had shown insufficient response to AZA therapy (100 mg daily, increased to 150 mg after two months; treatment duration six months), defined as <30% improvement of at least three or the four parameters mentioned. Group 3 consisted of patients who stopped AZA treatment within three months after the start because of adverse reactions.

CLINICAL ASSESSMENTS

Assessments during the double-blind trial have been reported in detail previously.⁵ For this study, parameters of disease activity were determined at the time blood was drawn for measurement of purine enzymes, and included RAI, number of swollen joints, patient's assessment of pain and general health on a visual analogue scale, and ESR. The disease activity score (DAS) was calculated.⁷

HEALTHY CONTROLS

The same purine enzymes were measured in peripheral blood cells of 14 age and sex-matched healthy volunteers.

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LABORATORY ASSESSMENTS

Erythrocyte sedimentation rate (ESR, Westergren), whole blood count and liver and kidney function tests were obtained simultaneously with the measurement of purine enzyme activities. Lymphocyte activities of 5'-nucleotidase (5NT), purine nucleoside phosphorylase (PNP), hypoxanthine-guanine phosphoribosyltransferase (HGPRT) and adenosine phosphoribosyl-transferase (APRT) and erythrocyte activities of thiopurine methyltransferase (TPMT) were measured using micro-enzymatic radiochemical assays as described previously.^{8,9} The analyst was 'blind' for the clinical data at the time purine enzymes were measured. All assays were done in quadruplicate.

STATISTICAL ANALYSIS

Student's *t* test or Wilcoxon's two-sample test was used to compare purine enzyme activities between patients and healthy controls, between sexes and between patients with or without prednisone. Correlations between purine enzymes and disease activity parameters were assessed according to Pearson or Spearman.

Levels of purine enzymes in the three groups of patients were compared by one-way analyses of variance. In case of non-normality the Kruskal-Wallis test was applied, followed by pairwise Wilcoxon's tests, using the Bonferroni principle with $\alpha/3$ as the level of significance. Tests were two-sided with $\alpha = 0.05$. Patients with deficiencies of purine enzymes known to influence the response to AZA treatment, such as HGPRT and TPMT deficiency, were excluded from this part of the analysis.

Results

STUDY POPULATION

Thirty six patients with RA (table 1) and 14 healthy controls (six male, eight female; median age 51 years, range 42–64) were studied. Eight patients had never used AZA, nine could be assigned to group 1 (good response), seven to group 2 (insufficient response), and 12 to group 3 (adverse reactions). A variety of second-line antirheumatic drugs (SARDs) were being used at the time purine enzymes were measured (table 1). Of the nine patients in group 1, seven continued to use AZA at the time purine enzymes were measured, but in two AZA had had to be withdrawn in the meantime because RA had relapsed. Adverse reactions leading to AZA-withdrawal included GI discomfort ($n = 6$), elevated transaminases ($n = 4$), drug-fever ($n = 3$), pancytopenia ($n = 1$), and mild leucopenia ($n = 1$).

PURINE ENZYME LEVELS

Mean coefficient of variation within assays was 0.11, 0.18, 0.10, 0.07 and 0.03 for APRT, HGPRT, 5NT, PNP and TPMT, respectively. Activities of APRT, HGPRT, 5NT and PNP are expressed in $\text{nmol} \cdot 10^{-6} \text{lymphocytes} \cdot \text{hr}^{-1}$, TPMT activity in $\text{pmol} \cdot 10^{-6} \text{erythrocytes} \cdot \text{hr}^{-1}$. The one patient experiencing severe bone

Table 1 Characteristics of 36 patients with rheumatoid arthritis

female/male	22/14
age (years)	
median	60
range	27–75
rheumatoid factor positive (n)	28
ANA positive (n)	18
APF positive (n)	22
prednisone (+/-)	11/25
SARD actually used*	
SASP	3
Au im	6
MTX	9
AZA	7
CP	1
none	10

*At the time purine enzymes were measured; stable medication for at least 1 month. SASP = sulphasalazine; Au im = parenteral gold; MTX = methotrexate; AZA = azathioprine; CP = cyclophosphamide.

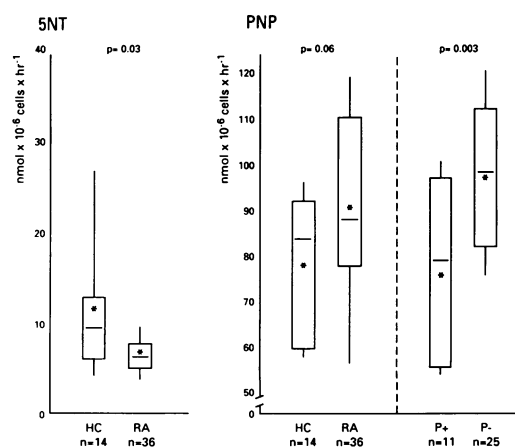
marrow depression had an undetectable low level of TPMT. No HGPRT or APRT deficiency was observed. For HGPRT, APRT and TPMT no differences between the groups studied were found.

PATIENTS WITH RA AND HEALTHY CONTROLS

Levels of 5NT activity were significantly lower in patients with RA than in healthy controls ($p = 0.03$) (figure). Ninety per cent of patients with RA had 5NT activity below the median of healthy controls. Levels of PNP tended to be higher in patients with RA than in healthy controls ($p = 0.06$). PNP activity was significantly lower in patients using prednisone than in those who did not ($p = 0.003$) (figure). No influence of prednisone was found on 5NT activity. Correlation coefficients for the association between enzyme activities and parameters of disease activity, including the DAS,⁷ did not exceed 0.39 (data not shown).

PATIENT GROUPS WITH A DIFFERENT RESPONSE TO AZA

No differences between the three groups were found in either sex distribution or the



Box plots of purine enzyme activities in patients with rheumatoid arthritis (RA) and healthy controls (HC). Left panel: 5'-Nucleotidase. Right panel: purine nucleoside phosphorylase; P+: patients with RA using prednisone, P-: patients with RA not using prednisone. (Each box represents the 25th to 75th percentile; the horizontal bar in each box represents the median value, the asterisk the mean. The vertical bar represents the distribution of the 10th and 90th percentile).

Table 2 Lymphocyte activities of 5NT and PNP in three groups of patients with RA with different responses to AZA treatment

	Good response (group 1; n = 9)	Insufficient response (group 2; n = 7)	Adverse reactions (group 3; n = 11)	p-value
5NT*				
75th percentile	9.7	8.6	7.5	
median	7.6	5.1	5.5	0.02†
25th percentile	7.4	4.0	4.8	
PNP				
mean (SD)	102.5 (19.4)	83.8 (21.6)	82.7 (22.9)	0.11‡

*Enzyme activity expressed in nmol·10⁹ lymphocytes·hr⁻¹.
†Kruskal-Wallis test. ‡One way ANOVA.

proportion of patients using prednisone. The overall hypothesis of equal levels of 5NT activity in the three response groups had to be rejected ($p = 0.02$) (table 2). A significantly higher mean was found for good responders than for patients with adverse reactions ($p = 0.012$; $\alpha = 0.017$), and an almost significantly higher mean compared with insufficient responders ($p = 0.03$). No difference between group 2 and group 3 was found ($p = 0.79$; $\alpha = 0.017$) (table 2).

Discussion

This is the first report of a possible association of purine enzyme activities with the outcome of AZA treatment in RA. The parallel between thiopurine metabolism and endogenous purine pathways suggests that the levels of intracellular purine enzymes are important determinants of the effects of AZA treatment. This is supported by the observation that purine enzyme deficiencies may reduce its efficacy,¹ or increase toxicity.^{2,3} In addition, the observation that levels of some purine enzymes were different in healthy controls and patients with RA,¹⁰ suggests that further study of purine enzymes in RA may be rewarding. Despite the retrospective design of this pilot study, and the lack of pretreatment values of purine enzymes, our data point to a possible role of purine enzymes in the response to AZA treatment in RA.

We found that lymphocyte activity of 5NT is lower in patients with RA than in healthy controls (figure). This is in agreement with the results of Appelboom *et al.*¹⁰ In addition, in our study PNP activity tends to be higher in RA than in healthy controls, and seems to be influenced by the use of prednisone. At high doses glucocorticoids cause a temporary lymphocyte depletion in the peripheral circulation, affecting T-lymphocytes more strongly than B-lymphocytes,¹¹ the latter having higher levels of PNP activity than T cells.¹² Therefore, if the same would apply for the lower doses used in our patients, prednisone would be expected to cause an increase instead of a decrease in PNP levels measured in unfractionated peripheral blood lymphocytes.

The reason why PNP and 5NT levels in patients with RA differ from levels in healthy controls remains unclear, but may relate to either the disease itself or the drugs used to treat it. Correlations between purine enzyme activities and parameters of disease activity were not impressive in our study. Admittedly,

at the time purine enzymes were measured, a variety of SARDs were being used in this group of patients. However, currently no data exist on a possible influence of SARDs on purine enzymes. Although it is tempting to assume that disturbances in purine enzymes play a part in the pathogenesis of RA, at present insufficient data are available to address this issue.

Our observations in the three groups of patients with different responses to AZA therapy are of special interest. Generally, cytotoxic thionucleotides are held responsible for both the favourable and toxic effects of AZA, suggesting that purine enzymes determine the effects of AZA by directly influencing the levels of cytotoxic metabolites. If this were to be the underlying mechanism, activities of catabolic enzymes, such as 5NT, would be expected to be low both in good responders and in patients experiencing adverse reactions, and higher in those who lack efficacy. Contrary to this relatively simple model, we found higher lymphocyte activities of 5NT in good responders than in both other groups. Perhaps, a presently not well understood more delicate balance between purine enzyme activities and products of (thio-) purine metabolism determining the final outcome of AZA treatment in RA must be considered. Prospective studies are needed to confirm our findings and will help in a better understanding of the mode of action of AZA in RA.

In conclusion, this study indicates that purine enzyme activities in patients with RA differ from healthy controls, may be associated with the outcome of AZA treatment, and seem not to be associated with disease activity.

The aetiopathogenetic and clinical implications of these findings need to be clarified. We believe that the possible identification of a factor predicting the response to AZA treatment in patients with RA merits the efforts of prospective studies.

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- 1 Kelley W N, Rosenbloom F M, Seegmiller J E. The effects of azathioprine (Imuran) on purine synthesis in clinical disorders of purine metabolism. *J Clin Invest* 1967; **46**: 1418-529.
- 2 Lennard L, Van Loon J A, Weinsilboum R M. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurinemethyltransferase genetic polymorphism. *Clin Pharmacol Ther* 1989; **46**: 149-54.
- 3 Kerstens P J S M, Stolk J N, Hilbrands L B, van de Putte L B A, De Abreu R A, Boerbooms A M T. 5-Nucleotidase and azathioprine-related bone-marrow toxicity. *Lancet* 1993; **342**: 1245-6.
- 4 Ropes M W, Bennet G A, Cobb S, Joco R, Jessor R A. Diagnostic criteria for rheumatoid arthritis: 1958 revision. *Ann Rheum Dis* 1959; **18**: 49-53.
- 5 Jeurissen M E C, Boerbooms A M T, van de Putte L B A, *et al.* Methotrexate versus azathioprine in the treatment of rheumatoid arthritis. A forty-eight-week randomized double-blind trial. *Arthritis Rheum* 1991; **34**: 761-72.
- 6 Ritchie D M, Boyle J A, McInnes J N, Greiveson P, Buchanan W. Clinical studies with an articular index for assessment of joint tenderness in patients with rheumatoid arthritis. *Q J Med* 1968; **37**: 393-97.
- 7 van der Heijde D M F M, van't Hof M A, van Riel P L C M, *et al.* Judging disease activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score. *Ann Rheum Dis* 1990; **49**: 916-20.
- 8 van Laarhoven J P R M, Spierenburg G T H, de Bruyn C H H M. Enzymological analysis of purine metabolism in lymphoid cells. *J Immunol Methods* 1980; **39**: 47-58.

- 9 Weinsilboum R M, Raymond F A, Pazonino P A. Human erythrocyte thiopurine methyltransferase: radiochemical microassay and biochemical properties. *Clin Chim Acta* 1978; **85**: 323-33.
- 10 Appelboom T, Mandelbaum I, Vertongen F. Purine enzyme levels in rheumatoid arthritis. *J Rheumatol* 1985; **12**: 1075-8.
- 11 Fauci A S, Dale D C, Balow J E. Glucocorticosteroid therapy: mechanisms of action and clinical considerations. *Ann Intern Med* 1976; **84**: 304-15.
- 12 van Laarhoven J P R M, Spierenburg G T, Collet H, Delespesse G, de Bruyn C H M M. Purine interconversion pathways in T, B, T γ and T-T γ cells from human peripheral blood. *Adv Exp Med Biol* 1984; **165B**: 111-8.