

PYRIMIDINE 5'NUCLEOTIDASE I AND II ACTIVITIES IN ACUTE LEUKAEMIAS

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Summary: Background and objective: Pyrimidine 5'nucleotidase I and II activities of peripheral mononuclear cells were studied to evaluate their role in diagnosis, assessment of therapy and follow up of remission in acute leukaemias. Design and methods: Blood samples were obtained from 40 untreated patients with acute lymphoblastic and myeloid leukaemia and 40 healthy controls, before the therapy and after remission. The correlation between the activity of the enzymes and the efficacy of therapy were established. The enzyme activities were measured by High-Performance Liquid Chromatography (HPLC), using the method described by Amici. For statistical analysis, Mann-Whitney U, Kruskal-Wallis and Wilcoxon methods were used. Results: Before the therapy, Pyrimidine 5'nucleotidase I levels in the leukaemic group were found to be significantly elevated when compared to the control group ($p < 0.001$). Also Pyrimidine 5'nucleotidase II levels were significantly elevated before the therapy and during remission ($p < 0.02$ and $p < 0.001$ respectively). The iso-enzyme activities were compared in patients who were in remission, who did not respond to therapy and in patients who died during the therapy, but no significant difference was found. Interpretation and conclusions: We concluded that, Pyrimidine 5'nucleotidase I and II activities can be used as markers for diagnosis and follow up of remission in patients with acute leukaemia. But, they can not have predictive value for prognosis.

Key words: *Pyrimidine 5'nucleotidase isoenzymes; Leukaemia*

Introduction

One additional approach to identification of malignant haematopoietic cells that has already shown diagnostic and therapeutic promise is that of purine pathway enzyme quantitation (9).

5' nucleotidase (5'NT) is one of the enzymes most extensively studied in this field which exists both on the cell membrane and in the cytosol (13). Since nucleotides are the proximate precursors for RNA and DNA replication, the level of 5'NT activity in the cells is apparently important for DNA replication, on which the cell proliferation depends (12). The enzyme activity appears to be higher in tissues in active DNA synthesis or with a higher turnover rate of nucleic acids and their precursors (7). The isoforms of 5'NT; pyrimidine 5'nucleotidase I and II enzymes (PN I and PN II) have different characteristic features referring to specificity for substrates and kinetic behaviors. Several investigators have shown increased or decreased 5' NT activities among subclasses of leukaemia in untreated patients or patients being treated (5,10).

In this paper we examined the role of PN I and PN II activities in diagnosis, assessment of therapy and follow up of remission in acute leukaemias by measuring enzyme activities prior to therapy and after remission.

Methods and materials

The study population consisted of 40 untreated patients (20 females, 20 males; mean age 44.2 years, range 16-78 years) with acute lymphoblastic leukaemia and acute myeloid leukaemia, in whom the diagnosis was made by cytological and histochemical investigations of peripheral blood and bone marrow smears. Eighty percent of the patients had AML and the other patients were with ALL (20%). The patients had a median age of 44.2 ± 18.6 years (range: 16-78) and 50% of the patients were female. Forty healthy adult subjects (21 males, 19 females; mean age 42.9, range 16-78 years) served as controls. Venous blood samples were obtained from patients and control group (prior to therapy and during first remission) and drawn into sodium heparin containing tubes. The mononuclear cells were isolated from the samples using the gradient centrifugation method described by Boyum et al. (3), were then stored at -20 °C until assayed. PN I and PN II activities were measured by the method described by Amici et al. (2), which was a procedure described for developing HPLC-based assays for nucleotidases by using a 20-mm C-18 reversed-phase column. Enzyme activities were detected using isocratic conditions which separate the product (nucleoside) from the substrate (nucleotide) in less than 1 min with 100 mM potassium

phosphate, pH 6.0, at a 2 ml/min flow rate. Nucleoside products of the enzymatic reactions were directly quantitated from their ultraviolet absorbances (2). Mann-Whitney U, Kruskal-Wallis and Wilcoxon tests were used for the comparison of activities between the groups. Spearman Rank test was used for correlation between the groups. $P < 0.05$ was accepted to be statistically significant.

Results

Eighty percent of the patients had AML and the other patients were with ALL (20%). Eight patients were M0, 6 patients were M2, 5 patients were M3, 9 patients M4, 3 patients were M5 and 1 patient was M6 according to French-American-British (FAB) criteria. Four of the patients were pre-B-ALL, 1 of the patients was B-ALL and 3 of them were T-ALL.

After the induction therapy 23 patients developed complete remission (57.5%) while 13 patients (32.5%) did not. Four patients were receiving therapy while the study ended. While 6 of the patients were on post-remission therapy; 4 patients died because of infections and 2 patients died because of thrombocytopenic hemorrhages.

As given in Table 1; PN I activity of patients before treatment (mean 1074.3, range 52.2–4158.1 U/mg protein) was significantly higher than those in healthy controls (mean 368.3, range 56.5–1749.7 U/mg protein). Also PN II activity of patients prior to therapy (mean 1043.8, range 53.8–4048.2 U/mg protein) was significantly elevated compared with those in the control group (mean 474.8, range 53.3–1305.2) ($p < 0.02$).

When compared between patient and control groups, PN II activity after remission was significantly lower in the control group than the patients (mean 474.8, range 53.3–1305.2 and mean 1789.6, range 617.4–5794.6 for controls and patients respectively, $p < 0.001$), while PN I activities was not. PN I and PN II activities of patients after remission were significantly different than activities prior to therapy ($p < 0.03$ and $p < 0.05$ respectively).

The isoenzyme activities were compared in patients who were in remission, who did not respond to therapy and in patients who died during the therapy, but no significant difference was found.

Discussion

In addition to traditional haematological methods as morphology and cytochemistry, highly specific techniques are required nowadays for the identification and sophisticated characterization of malignant and normal haematological cells. There have been many exciting developments in the clinical utility of biochemical enzyme markers for both diagnostic and therapeutic purposes in acute leukaemia (4,5,6,8,14).

5'NT activity of leukaemic cells have been the topic of several studies. This enzyme catalysis the dephosphorylation of 5' nucleotides and exists on the membranes of human peripheral-blood lymphoid cells. In some studies enzyme activity was measured in erythrocytes, T and B-lymphocytes, mononuclear and plasma cells (6,8,14). In our study 5' NT activity was determined in mononuclear cells. As the analysis of enzymatic methods are different from previous studies we were unable to discuss our results with them (2,6). We preferred to use HPLC, as the method is 50-fold more sensitive than that based on calorimetric measurements and furthermore avoids the use of radioactive materials (2).

Previous studies have determined enzyme activities in untreated patients or in patients being treated. This was the first study evaluating the role of PN I and PN II activities in diagnosis, assessment of therapy and follow up of remission in acute leukaemias by measuring enzyme activities prior to the therapy and after remission. As this is the first study we were not able to compare our results with previous literatures.

Previous literature have reported normal or decreased levels of 5' NT activity in the cells of leukaemia patients while being treated (4,5,6,10,11). But in our study PN I and PN II activities were higher than the control group which was statistically meaningful ($p < 0.001$ and $p < 0.02$ respectively). After remission a decrease in PN I activities were seen to levels of controls. PN II activities before treatment were twice the control activities and after treatment increased to four-fold activity of controls. Increase levels of PN I and PN II activities in leukaemia patients indicates that they could be used as markers in diagnosis of acute leukaemia. According to these results the decrease of PN I and increase of PN II after therapy could be used as markers of remission.

Tab. 1: Pyrimidine 5'-nucleotidase activities in control group and patients before therapy and after remission.

	PN I*			PN II*		
	Controls	Patients		Controls	Patients	
		Before therapy	After remission		Before therapy	After remission
Mean	368.3 ^a	1074.3 ^{a,d}	354.9 ^d	474.8 ^{b,c}	1043.8 ^{b,c}	1789.6 ^{c,e}
Minimum	56.5	52.2	94.0	53.3	53.8	617.4
Maximum	1749.7	4158.1	816.0	1305.2	4048.2	5794.6

PN I: Pyrimidine 5'-nucleotidase I, PN II: Pyrimidine 5'-nucleotidase II

*U/mg protein, ^a $p < 0.001$, ^b $p < 0.023$, ^c $p < 0.001$, ^d $p < 0.03$, ^e $p < 0.05$

Agostinho AB et al. reported that 5' NT activity was the summation of the activities of ecto 5' NT, PN I and PN II (1). Ecto 5' NT is found on the surface of lymphocytes and regulates intracellular penetration of nucleotides as nucleosides (8,11). In the cytosol of the same cells, two isoforms of 5' NT have been described; PN I and PN II. In leukaemia patients ecto 5' NT activity have been found to be normal or decreased in previous studies. In our study the decrease in PN I and increase in PN II activities after treatment indicates that; decrease in total 5' NT in previous studies was related to decreases in ecto 5' NT and PN I. The increase in PN I and PN II activities proves the increased turnover of DNA in acute leukaemia and new therapeutic agents inhibiting these enzymes may have clinical importance.

There was no significant difference between enzyme activities of patients who were in remission, who did not develop remission and who died ($p>0.05$). These results show that PN I and PN II activities do not have predictive value in determining prognosis.

Conclusions

According to these results PN I and PN II activities can be used as markers of diagnosis and follow up of remission in acute leukaemia but as a range of activity is found to be extremely wide, these tests diagnostic value is low in acute leukaemic patients. A study on a larger group should be performed in order to prove these results.

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