

Short communication

³¹P MRS ANALYSIS OF THE PHOSPHOLIPID COMPOSITION OF THE PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) AND BONE MARROW MONONUCLEAR CELLS (BMBC) OF PATIENTS WITH ACUTE LEUKEMIA (AL)

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Abstract: The aim of this study was to evaluate the phospholipid concentration in acute leukemia (AL) blast cells from peripheral blood (PBMC) and bone marrow (BMBC). *In vitro* ³¹P Nuclear Magnetic Resonance Spectroscopy (³¹P MRS) was used. The integral intensities of the resonant peaks and the phospholipid concentrations in PBMC and BMBC were analyzed. Differences in the phospholipid concentrations in cells from myeloblastic or lymphoblastic lines were also evaluated. This investigation was carried out on phospholipid extracts from PBMC and BMBC from 15 healthy volunteers and 77 patients with AL (samples taken at the moment of diagnosis). A significant decrease in sphingomyelin (SM) and phosphatidylserine (PS) was observed in the PBMC of patients with AL relative to the results for the healthy volunteers. For ALL, we

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Abbreviations used: AL – acute leukemia; ALL – acute lymphoblastic leukemia; AML – acute myeloblastic leukemia; BMBC – bone marrow mononuclear cells; CL – cardiolipin; CPLAS (PAF) – phosphatidylcholine plasmalogen; FAB – French-American-British classification; MDPA – methylenediphosphonic acid; MRS – magnetic resonance spectroscopy; PBMC – peripheral blood mononuclear cells; PC – phosphatidylcholine; PS – phosphatidylserine; PI+PE – phosphatidylinositol + phosphatidylethanolamine; SM – sphingomyelin

found a significant decrease in the concentration of phosphatidylcholine plasmalogen (CPLAS), SM, PI+PE (phosphatidylinositol + phosphatidylethanolamine) and PS in comparison with the results for healthy volunteers and patients with AML. Experiments with BMMC cells revealed a significant decrease in the concentration of CPLAS, SM, PI+PE, and PS in ALL relative to AML. Additionally, a significant decrease in phosphatidylcholine (PC) concentration was observed in ALL compared to AML. If the phospholipid extracts were taken simultaneously from the same patient, there were no significant differences in the integral intensities and phospholipid concentrations between PBMC and BMMC.

Key words: Acute leukemia, ^{31}P MRS, Phospholipids

INTRODUCTION

Establishing the concentration of phospholipids in malignant cells and their role in apoptosis is the aim of a great deal of research. To continue our previous studies on the application of ^{31}P MRS to the analysis of phospholipid changes in the sera [1-6], plasma [7], and peripheral blood and bone marrow mononuclear cells [8-10] from patients with blood cancer diseases, we decided to evaluate phospholipid concentrations in acute leukemia cells from the peripheral blood (PBMC) and bone marrow (BMMC). We also evaluated differences in the phospholipid concentrations in blast cells from myeloblastic and lymphoblastic lines. The *in vitro* ^{31}P MRS method was applied. On the basis of the received ^{31}P spectra of the phospholipid extracts from those cell types, the integral intensities of the resonant peaks for phospholipids and methylenediphosphonic acid (MDPA) were analyzed, and thus, the phospholipid concentrations could be calculated.

MATERIALS AND METHODS

This investigation was carried out on phospholipid extracts from the PBMC of 15 healthy volunteers and 55 patients with acute leukemia (18 with ALL and 37 with AML) and from the BMMC of 44 patients with acute leukemia (11 with ALL and 29 with AML). Experiments were also carried out on extract couples obtained simultaneously from the peripheral blood and bone marrow (PBMC/BMMC AL_{P+B}) of 13 patients with acute leukemia (Tab. 1).

The phospholipid extracts were obtained using the modified Folch method. ^{31}P NMR spectra were received on an AMX 300 Bruker (7.05 T) spectrometer. All the applied methods in this investigation were described in previous publications [7, 8, 10].

Tab. 1. Investigated groups.

Group		All	Females	Males	Age (Median)
PBMC	Z _p	15	6	9	22-55 (24)
	AL _p (= ALL _p +AML _p)	55	21	34	17-76 (49)
	ALL _p	18	5	13	17-62 (27)
	AML _p	37	16	21	30-76 (58)
	M _{2p}	12	6	6	30-76 (60)
	M _{4p}	15	6	9	36-74 (61)
BMMC	AL _B (= ALL _B +AML _B)	40	18	22	22-79 (56)
	ALL _B	11	4	7	23-76 (29)
	AML _B	29	14	15	22-79 (57)
PBMC	AL _p + AL _B	13	5	8	22-55 (25)
BMMC					

Z_p – healthy volunteers, AL_p – acute leukemia patients from whom PBMC were obtained, ALL_p – ALL patients from whom PBMC were obtained, AML_p – AML patients from whom PBMC were obtained, M_{2p} – AML M2 patients from whom PBMC were obtained, AL_B – acute leukemia patients from whom BMMC were obtained, ALL_B – ALL patients from whom BMMC were obtained, AML_B – AML patients from whom BMMC were obtained, AL_{p+B} – acute leukemia patients from whom PBMC and BMMC were obtained simultaneously.

RESULTS AND DISCUSSION

The ³¹P NMR spectra of the phospholipid extracts from the PBMC of the healthy volunteers (Z_p) consisted of 7 resonant peaks due to phospholipids – PC, CPLAS, SM, PI+PE, PS, and CL (cardiolipin) – and one other due to MDPA, the external reference substance. The resonant peak due to CL was only observed in 6 of 15 individuals. The ³¹P NMR spectrum of the phospholipid extracts from the PBMC of the healthy volunteers is shown in Fig. 1.

The phospholipid concentrations in the investigated extracts are based on the integral intensities of the resonant peaks due to phospholipids and MDPA (Tab. 2). A significant decrease in the integral intensities of the resonant peaks due to SM and PS was observed in the spectra of the phospholipid extracts from the PBMC of the AL_p group patients (Fig. 1). Additionally, the peak due to CPLAS was not observed in 25.4% of patients, that due to SM in 25.4%, that due to PI+PE in 3.6%, that due to PS in 32.7%, and that due to CL in 89.1%. The concentrations of SM and PS were significantly decreased in comparison with the Z_p group. The concentration decrease for PS was 66.7%, and for SM was 50.0%.

The spectra of the phospholipid extracts from PBMC were compared between patients with ALL (ALL_p group) and with AML (AML_p group). A significant decrease in the resonant peaks due to CPLAS, SM, PI+PE and PS was observed in the spectra for the ALL_p group patients in comparison with those for the AML_p group patients (Fig. 2). Similar differences were observed for phospholipid concentrations in the groups mentioned above (Tab. 2).

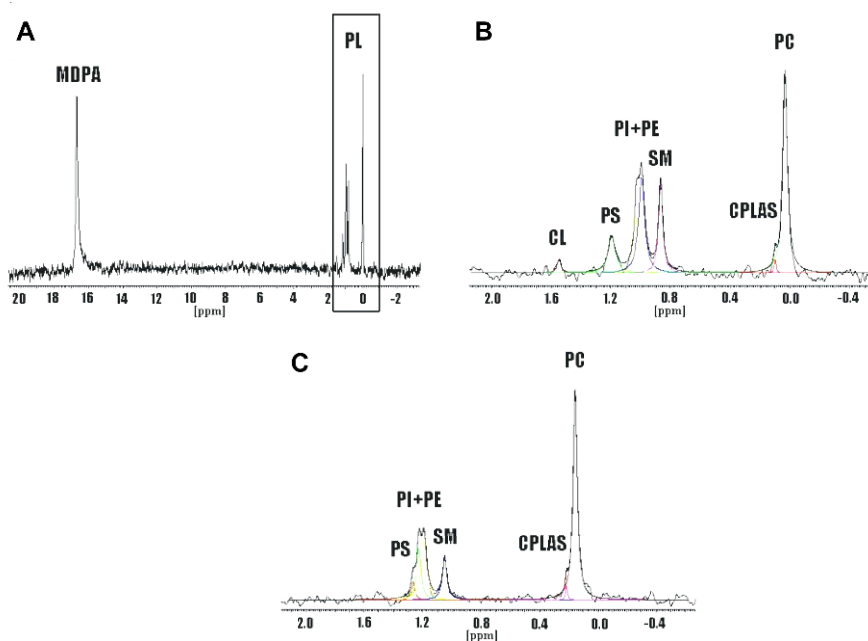


Fig. 1. The ^{31}P NMR spectra of phospholipid extracts from PBMC: PL in reference to MDPA (A), for healthy volunteers (B) and for patients with AL (C).

Additionally, the peak due to CPLAS was not observed in 44.4% of the ALL_P patients, that due to SM in 33.3%, that due to PI+PE in 5.6%, that due to PS in 50.0%, and that due to CL in 94.4%. The concentrations of phospholipids mentioned above for the ALL_P group were significantly decreased relative to those for the Z_P group.

The peak due to CPLAS was not observed in 16.2% of patients with AML_P , that due to SM in 21.6%, that due to PI+PE in 2.7%, that due to PS in 24.3%, and that due to CL in 84.5%. The concentrations of SM and PS in the extracts from PBMC were significantly diminished in the AML_P group relative to those for the Z_P group (Tab. 2). There were no significant differences in the phospholipid concentrations in the PBMC extracts between M_{2P} and M_{4P} according to the FAB (French-American-British Classification) of Acute Leukaemia (Tab. 2). The ^{31}P NMR spectra of phospholipid extracts from the BMMC of AL patients (AL_B group) at the moment of diagnosis consisted of 7 resonant peaks due to the phospholipids PC, CPLAS, SM, PI, PE, PS, and CL, and another due to MDPA (as with the spectra of PBMC) (Fig. 3). There was no peak due to CPLAS in 30.0% of patients, none due to SM in 25.0%, none due to PI+PE in 7.5%, none due to PS in 40.0%, and none due to CL in 75.0%. The phospholipid concentrations in the BMMC extracts of the AL_B , ALL_B , AML_B groups are shown in Tab. 2.

Tab. 2. The phospholipid concentrations in the investigated groups.

Phospholipids C (mmol/l)	PC	CPLAS	SM	PI+PE	PS	CL
Z _p n = 15	0.41 ± 0.03	0.04 ± 0.01	0.15 ± 0.01	0.29 ± 0.04	0.09 ± 0.01	0.03 ± 0.01
AL _p n = 55	0.37 ± 0.03	0.04 ± 0.01	0.07 ± 0.01	0.23 ± 0.02	0.03 ± 0.01	0.01 ± 0.00
ALL _p n = 18	0.31 ± 0.04	0.01 ± 0.01	0.05 ± 0.01	0.14 ± 0.02	0.01 ± 0.00	0.00 ± 0.00
AML _p n = 37	0.40 ± 0.03	0.05 ± 0.01	0.09 ± 0.01	0.27 ± 0.03	0.04 ± 0.01	0.01 ± 0.01
M ₂ n = 12	0.44 ± 0.07	0.05 ± 0.01	0.07 ± 0.02	0.27 ± 0.06	0.04 ± 0.02	0.00 ± 0.00
M ₄ n = 15	0.42 ± 0.03	0.05 ± 0.01	0.11 ± 0.01	0.29 ± 0.03	0.04 ± 0.01	0.01 ± 0.01
AL _B n = 40	0.34 ± 0.03	0.04 ± 0.01	0.08 ± 0.01	0.23 ± 0.02	0.00 ± 0.01	0.01 ± 0.00
ALL _B n = 11	0.23 ± 0.05	0.01 ± 0.01	0.03 ± 0.02	0.14 ± 0.04	0.01 ± 0.01	0.00 ± 0.00
AML _B n = 29	0.39 ± 0.03	0.05 ± 0.01	0.11 ± 0.01	0.26 ± 0.02	0.04 ± 0.01	0.02 ± 0.01
PBMC/BMMC						
AL _p n = 13	0.37 ± 0.05	0.05 ± 0.01	0.08 ± 0.02	0.26 ± 0.04	0.04 ± 0.01	0.02 ± 0.01
AL _B	0.33 ± 0.04	0.03 ± 0.01	0.07 ± 0.02	0.21 ± 0.03	0.03 ± 0.01	0.01 ± 0.01
			<i>p</i> - level			
Z _p :AL _p	NS	NS	<i>p</i> < 0.001	NS	<i>p</i> < 0.001	NS
ALL _p :AML _p	NS	<i>p</i> < 0.002	<i>p</i> < 0.020	<i>p</i> < 0.002	<i>p</i> < 0.040	NS
Z _p :ALL _p	NS	<i>p</i> < 0.002	<i>p</i> < 0.001	<i>p</i> < 0.003	<i>p</i> < 0.001	NS
Z _p :AML _p	NS	NS	<i>p</i> < 0.02	NS	<i>p</i> < 0.002	NS
M _{2p} :M _{4p}	NS	NS	NS	NS	NS	NS
ALL _B :AML _B	<i>p</i> < 0.007	<i>p</i> < 0.001	<i>p</i> < 0.002	<i>p</i> < 0.003	<i>p</i> < 0.001	NS
PBMC/BMMC						
AL _p :AL _B	NS	NS	NS	NS	NS	NS

Z_p – healthy volunteers, AL_p – acute leukemia patients from whom PBMC were obtained, ALL_p – ALL patients from whom PBMC were obtained, AML_p – AML patients from whom PBMC were obtained, M_{2p} – AML M2 patients from whom PBMC were obtained, AL_B – acute leukemia patients from whom BMMC were obtained, ALL_B – ALL patients from whom BMMC were obtained, AML_B – AML patients from whom BMMC were obtained, AL_{p+B} – acute leukemia patients from whom PBMC and BMMC were obtained simultaneously.

A significant decrease in the integral intensities of the resonant peaks due to PC, CPLAS, SM, PI+PE, and PS and its concentrations was observed in the spectra for patients of the ALL_B group relative to those for the AML_B group (as with the spectra for the PBMC groups) (Fig. 4). Additionally, the resonant peak due to CPLAS was not observed in 81.8% of patients of the ALL_B group, that due to SM in 63.6%, that due to PI+PE in 18.2%, and that due to PS in 90.9%. There was no peak due to CL for any patient. The resonant peak due to CPLAS was not observed in 10.3% of AML_B patients, that due to SM in 10.3%, that due to PI+PE in 3.4%, that due to PS in 20.7%, and that due to CL in 65.5%.

The ³¹P NMR spectra of phospholipid extracts from PBMC and BMMC were compared. These spectra originated from the same patients, from whom samples were collected simultaneously (PBMC/BMMC: AL_p+AL_B). There were no significant differences between these groups in terms of the integral intensities of resonant peaks or phospholipid concentrations (Tab. 2).

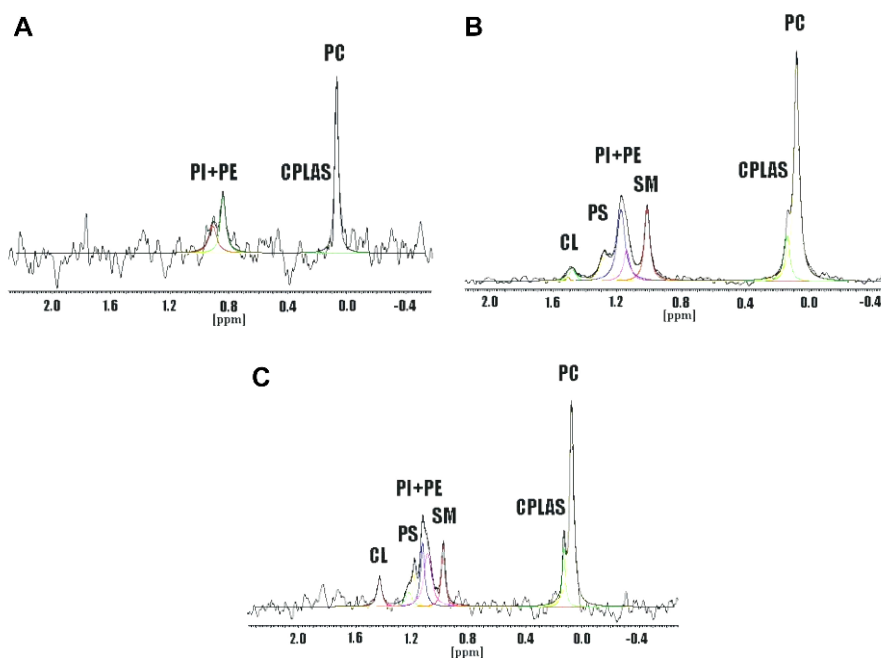


Fig. 2. The ^{31}P NMR spectra of phospholipid extracts from the PBMC of patients in the active stage of acute leukemia: lymphoblastic (A), myeloblastic M2 (B), and myeloblastic M4 (C) type.

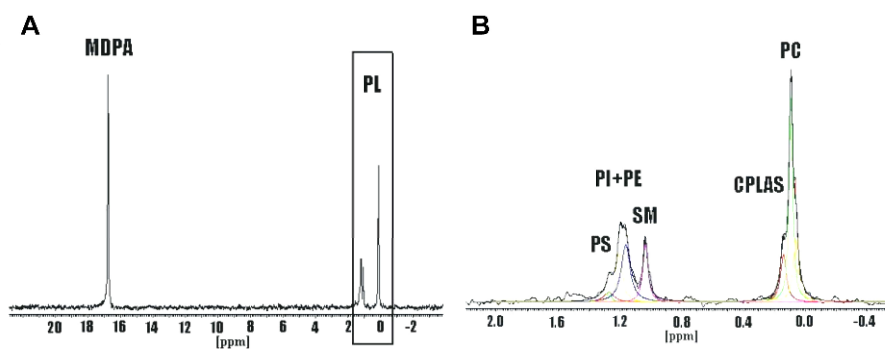


Fig. 3. The ^{31}P NMR spectra of phospholipid extracts from BMMC: PL in reference to MDPA (A), patients with AL (B).

Many sphingolipids, such as ceramide, sphingosine, sphingosine-1-phosphate (SP1), ceramide-1-phosphate and lyso-sphingomyelin, play regulatory roles in cell growth, death, adhesion, migration, inflammation, angiogenesis and intracellular signalling. On the basis of the data from the spectra of PBMC received from patients with AL (AML_P and ALL_P), it was claimed that the

integral intensities of resonant peaks due to SM and PS and the concentrations of these phospholipids are significantly diminished in comparison with those for healthy volunteers. Similar differences were observed by Merchant *et al.*, who investigated malignant changes in the tissues of the esophagus and colon [11, 12].

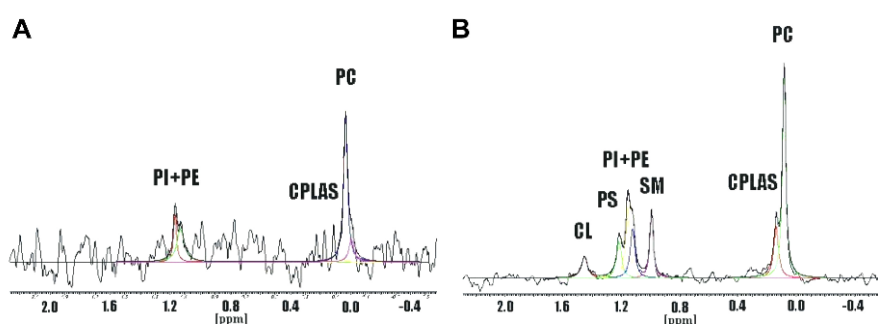


Fig. 4. The ³¹P NMR spectra of phospholipid extracts from the BMMC of ALL patients (A) and AML patients (B).

Eicosanoids, phosphoinositides, sphingolipids and fatty acids are signalling lipids which play an important role in cellular processes like cell proliferation, apoptosis, metabolism and migration. Cytokines, growth factors and nutrients initiate signal transduction events and modify the activity of lipid-modifying enzymes: phospholipases, prostaglandin synthase 5-lipoxygenase, phosphoinositide 3-kinase, sphingosine kinase and sphingomyelinase. Thus, the complex signalling network is initiated by multiple nodes of interaction, with cross-regulation where imbalance leads to disease. One such important lipid is sphingomyelin. This phospholipid is located in the outer leaflet of membranes. SM is present in a small amount in the inner leaflet. Sphingomyelinase causes SM hydrolysis into ceramide and phosphorylcholine. The role of ceramide in the initiation phase of apoptosis is probably to be the second mediator, while in the effectual phase, it takes part in apoptotic cell generation and membrane blebbing/vesiculation [13, 14]. On the basis of the aforementioned studies, it can be presumed that the deficiency of SM in the cell membranes of acute leukemic cells can cause a decrease in the ceramide concentration and influence the decrease in apoptotic cell death efficiency. It could be an additional factor for malignant cell maintenance and its accumulation in organisms.

In our investigation, the decrease in SM concentration was accompanied by a decrease in PS concentration. The disturbed amount of PS in the cell may cause disturbances of apoptotic processes. PS, which is located in the inner leaflet of cell membranes, gives a signal by externalization into the outer leaflet for phagocytes to recognize and remove apoptotic cells [15-20]. This decrease in PS level was observed also by Merchant *et al.* in malignant esophageal tissues [11].

We focused on the significant difference in the integral intensities and phospholipid concentrations of SM and PS as well as CPLAS and PI+PE between the ALL_P and AML_P groups. In the ALL_P group, these values were diminished in comparison with both the AML_P group and the healthy volunteers. In the AML_P group, only the levels of SM and PS were significantly decreased in comparison to those for the healthy volunteers. Similar observations were found in the phospholipid extracts from BMMC. For the patients with ALL_B, a significant decrease in PC, CPLAS, SM, PI+PE and PS was observed compared to patients with AML_B.

Studies concerning PE, especially lysophosphatidic acid acyltransferase-beta (LPAAT-beta), which takes part in lysophosphatidic acid acylation, could prove the existence of differences between the activity of this enzyme in ALL and AML. Upon the action of phospholipase, PE breaks up into ethanolamine and phosphatidic acid (PA). PA is also generated by lysophosphatidic acid acylation, which is catalyzed by lysophosphatidic acid acyltransferases (LPAATs). One of these acyltransferases is lysophosphatidic acid acyltransferase-beta (LPAAT-beta) [21]. The overexpression of this enzyme was proven in malignant changed ovaries [22, 23]. The increased overexpression of LPAAT-beta was not observed in the PBMC and BMMC of patients with AL and cell lines (AML193, Molt4, U937, CEM and K562) in comparison with healthy volunteers. It was proven that the level of this enzyme depends on the AL type. Increased activity was observed in ALL compared to AML. It was claimed that the cell sensitivity for the action of the LPAAT-beta inhibitor (CT-32228) is more increased in AML than ALL [24]. The increased demand for PA in ALL in comparison to AML and the possibility of receiving this phospholipid from PE can be the cause of the decrease in PI+PE observed by us in PBMC and BMMC from patients with ALL. Similar explanations can concern PC, which on the action of phospholipase D breaks up into choline and PA [25].

Data on the behavior of CPLAS in the PBMC and BMMC of acute leukemia was discussed by the authors of an earlier paper [9]. Differences in the lipid content in cell membranes were also observed between lympho- and myeloblastic cells from peripheral blood from children with acute leukemia [26]. We found differences in the structure of lymphoblastic membranes in comparison to leukocytes from healthy volunteers and in comparison to myeloblasts.

There are other methods which reveal differences in the apoptotic mechanisms of lympho- and myeloblastic cells. This was shown by Wu Lin *et al.* [27], who confirmed the increased activity of caspase-3 in PBMC from patients with AML in comparison with ALL. It can indicate an increased susceptibility of PBMC to apoptosis in AML in comparison to ALL [28]. These results concur with the data obtained by Invernizzi *et al.* [29], who observed the largest decrease of the amount of apoptotic cells in patients with ALL (1.0 ± 0.5) compared to patients with AML (8.6 ± 2.3), with MDS (16.7 ± 3.4) and healthy volunteers (10.8 ± 1.4).

The results of the abovementioned studies and our own observations indicate differences in the processes in the blast cells of lymphoblastic and myeloblastic lines. This can give opportunities to find new treatments for acute leukemia.

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