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Short communication

PLATELET-ACTIVATING FACTOR CHANGES IN PHOSPHOLIPID EXTRACTS FROM THE PLASMA, PERIPHERAL BLOOD MONONUCLEAR CELLS AND BONE MARROW MONONUCLEAR CELLS OF PATIENTS WITH ACUTE LEUKEMIA – A ^{31}P MRS *in vitro* STUDY

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Abstract: The aim of this investigation was to evaluate the changes in PAF concentrations in the plasma, PBMC and BMMC of patients with acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML). The plasma was from 23 healthy volunteers (HV) and 44 patients with AL (16 ALL, 28 AML). The PBMC were from 15 HV and 55 patients with AL (18 ALL, 37 AML), and the BMMC from 40 patients with AL (11 ALL, 29 AML). Methanol-chloroform phospholipid extraction from 60 x 10^6 cells (PBMC or BMMC) was performed according to a modified version of Folch's method. ^{31}P MRS data was obtained on an AMX 300 Bruker spectrometer (7.05 T). The PAF concentration in the plasma of the patients with ALL or AML was lower than that for the healthy volunteers. The PAF concentration in the plasma of the patients with ALL did not differ significantly from that of the patients with

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Abbreviations used: AL – acute leukemia; ALL – acute lymphoblastic leukemia; AML – acute myeloblastic leukemia; BMMC – bone marrow mononuclear cells; MRS – magnetic resonance spectroscopy; PAF (CPLAS) – platelet-activating factor, 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; PBMC – peripheral blood mononuclear cells

AML. In the case of both the PBMC and BMMC, the PAF concentration was significantly diminished in patients with ALL relative to the concentration for those with AML and for the healthy volunteers. No differences were observed in the PAF concentrations for the AML patients and the healthy volunteers.

Key words: PAF, Acute leukemia, ^{31}P MRS *in vitro*

INTRODUCTION

Phospholipids are major constituents of all biological membranes. Platelet-activating factor – PAF (CPLAS) – is an ether phospholipid compound with a wide range of activities towards blood cells [1, 2]. The chemical structure of PAF was determined to be 1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine [3, 4]. In nanomolar concentrations, this phospholipid plays a significant role in the activation of leukocytes and macrophages [5, 6], platelet aggregation [7], cell adhesiveness [8], angiogenesis [9] and other biological events.
In our last study, we applied ^{31}P MRS to analyze phospholipid changes in the plasma of patients with AL [10, 11], and to analyze the phospholipid composition of normal human PBMC [12]. The aim of this investigation was to evaluate the changes in PAF concentrations in the plasma, PBMC and BMMC of patients with acute lymphoblastic leukemia and acute myeloblastic leukemia.

MATERIALS AND METHODS

The plasma originated from 23 healthy volunteers (9 women and 14 men), aged from 22 to 55, and 44 patients with AL (18 women and 26 men), aged from 19 to 74. The AL plasma donors were divided into 2 groups: 16 patients with ALL, aged from 19 to 59, and 28 patients with AML, aged from 20 to 74. The PBMC were obtained from 15 healthy volunteers (6 women and 9 men), aged from 22 to 55, and 55 patients with AL (21 women and 34 men), aged from 17 to 76. The AL PBMC donors were divided into 2 groups: 18 patients with ALL, aged from 17 to 62, and 37 patients with AML, aged from 30 to 76. The BMMC were obtained from 40 patients with AL (18 women and 22 men), aged from 22 to 79. These BMMC donors were divided into 2 groups: 11 patients with ALL, aged from 23 to 76, and 29 patients with AML, aged from 22 to 79 (Tab. 1).
The diagnosis of the acute leukemia subtype was made on the basis of the morphological, cytochemical, cytogenetic and immunophenotypical features of the blast cells, and using the criteria of the French-American-British cooperative group (FAB). Phospholipid extraction was performed according to a modified version of Folch's method. The ^{31}P MRS spectra were obtained using an AMX Bruker 300 spectrometer. The methods were described previously in detail [10-12].
The chemical shift of the PAF peak in the ^{31}P NMR spectra of the investigated phospholipid extracts (from the plasma, PBMC or BMMC) equaled 0.14 ppm in

reference to 85% orthophosphoric acid. This peak was identified using a standard analogue of PAF (β-acetyl-γ-O-alkyl-L-α-phosphatidylcholine, from bovine heart lecithin, lyophilized powder), purchased from SIGMA.

Tab. 1. The numbers of individuals and median age in each of the study groups.

		Plasma		PBMC		BMMC	
		N	Age (median)	N	Age (median)	N	Age (median)
HV		23	25	15	24	0	-
ALL		16	26	18	27	11	29
	B	12	-	14	-	9	-
	T	4	-	4	-	2	-
AML		28	44	37	60	29	57
	M0	3	-	2	-	1	-
	M1	2	-	4	-	2	-
	M2	8	-	12	-	7	-
	M3	8	-	1	-	0	-
	M4	10	-	15	-	12	-
	M5	3	-	2	-	6	-
	M6	0	-	1	-	1	-

RESULTS AND DISCUSSION

The ^{31}P NMR spectra of the phospholipid extracts from the plasma are shown in Fig. 1. The spectra for the patients with AL demonstrated the reduction in integral intensity of the PAF peak in comparison with that for the healthy volunteers. Additionally, no PAF peak was observed in 18% of patients. There were no differences between the ALL and AML patients in terms of the integral intensity of the PAF peak (Fig. 1).

The ^{31}P NMR spectra of the phospholipid extracts from the PBMC are shown in Fig. 2. The integral intensity of the PAF peak for patients with ALL was decreased relative to that for patients with AML and healthy volunteers. No PAF peak was observed in 44% of patients with ALL. In the case of AML patients, the peak due to this phospholipid did not appear for only 16% of the patients.

Fig. 2 also shows the ^{31}P NMR spectra for the phospholipid extracts from the BMMC. The differences between the ALL and AML patients in the PAF peaks of these spectra were similar to those observed with the PBMC. The integral intensity of the PAF peak was also diminished in patients with ALL compared to that for patients with AML. No PAF peak was observed in 82% of patients with ALL. This peak was missing in 10% of patients with AML.

The integral intensity of the PAF peak in the ^{31}P NMR spectra permitted the evaluation of the concentration of PAF in the phospholipid extracts. The PAF concentrations in the above-mentioned phospholipid extracts are given in Tab. 2.

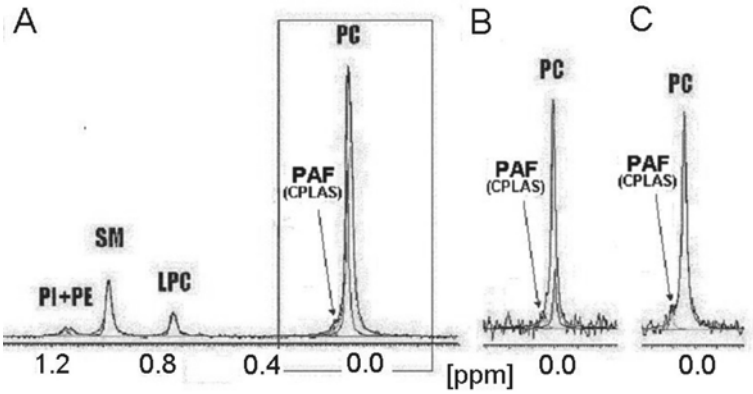

Fig. 1. The ^{31}P NMR spectra of phospholipid extracts from the plasma. A – healthy volunteers, B – patients with ALL and C – patients with AML.

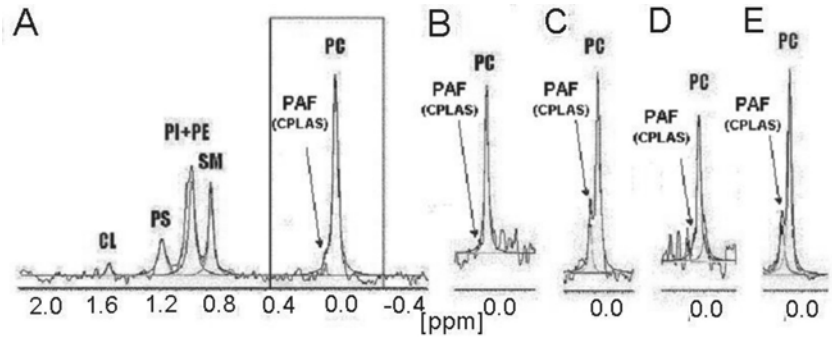

Fig. 2. The ^{31}P NMR spectra of phospholipid extracts. A – Extracts from the PBMC of healthy volunteers, B – patients with ALL, C – patients with AML. D – Extracts from the BMMC of patients with ALL and E – patients with AML.

Tab. 2. The PAF concentrations in phospholipid extracts from the plasma, PBMC and BMMC of the test subjects.

	Plasma			PBMC			BMMC	
	HV (n = 23)	ALL (n = 16)	AML (n = 28)	HV (n = 15)	ALL (n = 18)	AML (n = 37)	ALL (n = 11)	AML (n = 29)
C[mmol/l]	0.20±0.05	0.05±0.02	0.06±0.02	0.04±0.01	0.01±0.01	0.05±0.01	0.01±0.01	0.05±0.01
Level p	HV:ALL $p < 0.001$	ALL:AML NS	HV:AML $p < 0.001$	HV:ALL $p < 0.002$	ALL:AML $p < 0.002$	HV:AML NS	ALL:AML $p < 0.001$	

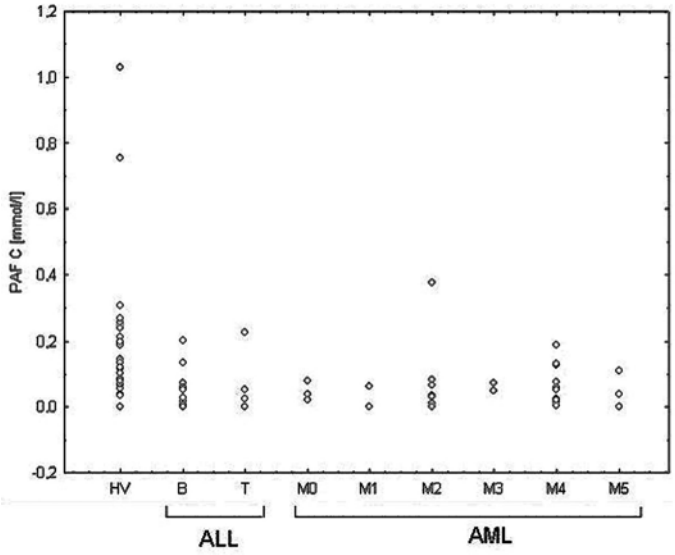

Fig. 3. The PAF concentrations in phospholipid extracts from the plasma of patients with AL.

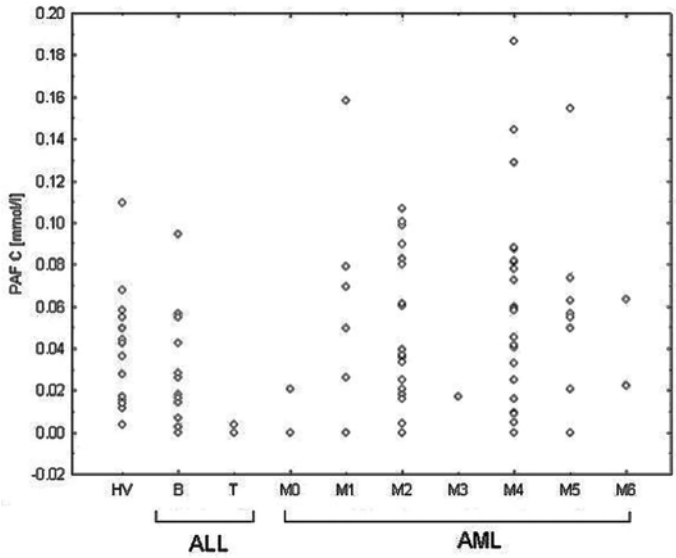

Fig. 4. The PAF concentrations in phospholipid extracts from the PBMC and BMMC of patients with AL.

The concentrations of PAF in the plasma of patients with ALL or AML appeared significantly reduced relative to those for healthy volunteers, but there was no significant difference between the concentrations for AML and ALL patients. In both the PBMC and BMMC, the PAF concentration was significantly diminished in patients with ALL relative to the concentration for those with

AML and for the healthy volunteers. No differences were observed in the PAF concentrations between AML patients and healthy volunteers. Subtypes of ALL and AML were also taken into consideration. The average concentrations of PAF in the phospholipid extracts from the plasma, PBMC and BMMC are given in Figs 3 and 4.

In our study, the PAF concentration in the plasma of patients with ALL or AML was reduced in comparison to that for the healthy volunteers. Moreover, no differences between ALL and AML patients in terms of the PAF concentrations in the plasma were observed. This result concurs with those reported by Denizot *et al.* [15], who analyzed the PAF levels in the blood and the acetylohydrolase (AHA) levels in the serum of 79 patients with lymphoid (NHL, HD, LLC, MM) and non-lymphoid (AML, RAEB, MLC, PRV) hematological malignancies. Lower blood PAF levels were found in all these patients than in healthy volunteers. Moreover, the AHA levels were not different between healthy controls and these patients. Those authors tried to find an explanation for these phenomena in terms of decreased PAF production by circulating and endothelial cells in patients with hematological cancers [15, 16].

The PAF activity in human bone marrow is higher (576 ± 39 pg/ml) than in the blood (374 ± 22 pg/ml). No correlation was found between the PAF amount and the lymphocyte, monocyte and erythroblast counts, but the PAF correlated with the granulocyte count [17]. It was observed that human marrow stromal cells produced 50-fold more PAF than freshly isolated BMMC, suggesting that stromal cells might be the major source of the human marrow-derived PAF [18]. Based on our previous investigations, we claimed that phospholipid concentrations from the PBMC do not differ significantly from those from the BMMC (Kuliszkiewicz-Janus M. *et al.* unpublished data). This has now been confirmed for the PAF concentration. It is very interesting that the concentration of PAF in the blood of the patients with ALL is significantly diminished in comparison with that for the patients with AML and the healthy volunteers. There was no difference between the patients with AML and the healthy volunteers in this case. A possible explanation for this observation should be found in the relationship between PAF contents in blast cells and the presence of membrane or putative intracellular PAF receptors (PAF-Rs) [16]. The results of Donnard *et al.* indicated no membrane PAF-Rs on the blast cells of all the investigated AML patients. By contrast, the blasts from 7 out of 15 ALL patients contained membrane PAF-Rs. Putative intracellular PAF-Rs were found in blasts of all the investigated ALL and AML patients [19]. This observation concerning the lack of membrane PAF-Rs in the case of AML of all the FAB subtypes was confirmed by Berdel *et al.* [20]. Moreover, membrane PAF-Rs could be detected in a histiocytic lymphoma line and in peripheral blood neutrophils and monocytes from healthy donors [20]. Additionally, the findings of Garcia *et al.* [21] showed the ability of myeloid cell lines (HL60 and U937) to synthesize PAF, in contrast to the absence of this property in the two lymphoid cell lines (Daudi and Jurkat). The clinical studies by Gugliemi *et al.* highlight

membrane PAF-R in several types of chronic mature B-cell malignancies: chronic lymphocytic leukemia [22], mantel B-cell lymphoma, marginal zone B-cell lymphoma, plasma cell lymhoma, prolymphocytic or prolymphocytoid B-cell leukemia, and follicular B-cell lymphoma [23]. No presence of this receptor was observed on leukemic blasts of patients with acute B-lymphoid leukemia [19]. Thus, the hypothesis arises that the expression of membrane PAF-R is a marker of B-cell differentiation and maturation. In this study, we noticed a higher PAF concentration in more mature cells, i.e. AML M2, M4 and M5, than in the poorly differentiated M0 and M1. The insufficient number of patients with individual FAB subtypes was a major cause of difficulty for valid statistical data analysis in the individual groups. Similar results were observed by Foa *et al.* [24], who suggested that human leukemic cells of lymphoid and myeloid origin show different capacities of releasing PAF possibly due to the level of differentiation of the cells.

The most recent study of Reynaud *et al.* [25] shows the functional presence of PAF-R in the blast cells of patients with acute leukemia, a result that could be of physiological importance regarding the effect of PAF on leukocyte maturation and function. These results provide the foundation for the study of the role of PAF in regulating human blast cell proliferation and apoptosis, an area which requires further investigation.

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REFERENCES

1. Mazer, B.D., Sawami, H., Franklin, R. and Gelfand E.W. B-cell activation and regulation of immunoglobulin synthesis by platelet activating factor. **Neth. J. Med.** 39 (1991) 224-253.
2. Rola Pleszczynski, M., Pouliot, C., Turcotte, S., Pignol, B., Braquet, P. and Bouvrette, L. Immune regulation by platelet-activating factor: I. induction of supressor cell activity in human monocytes and CD8+ T cells and of helper cell activity in CD4+ T cells. **J. Immunol.** 140 (1988) 3547-3552.
3. Demopoulos, C.A., Pinckard, R.N. and Hanahan, D.J. Platelet-activating factor. Evidence for 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine as the active component (a new class of lipid chemical mediators). **J. Biol. Chem.** 254 (1979) 9355-9358.
4. Blank, M.L., Snyder, F., Byers, L.W., Brooks, B. and Muirhead, E.E. Antihypertensive activity of an alkyl ether analog of phosphatidylcholine. **Biochem. Biophys. Res. Commun.** 90 (1979) 1194-1200.
5. O'Flaherty, J.T., Miller, C.H., Lewis, J.C., Wykle, R.L., Bass, D.A., McCall, C.E., Waite, M. and De Chatelet, L.R. Neutrophil responses to platelet-activating factor. **Inflammation** 5 (1981) 193-201.
6. Prpic, V., Uhing, R.J., Weiel, J.E., Jakoi, L., Gawdi, G., Herman, B. and Adams, D.O. Biochemical and functional responses stimulated by platelet-

activating factor in murine peritoneal macrophages. **J. Cell. Biol.** 107 (1988) 363-372.
7. Chignard, M., Le Couedic, J.P., Tence, M., Vargftig, B.B. and Benveniste, J. The role of platelet-activating factor in platelet aggregation. **Nature** 279 (1979) 799-800.
8. Mc Intyre, T.M., Zimmerman, G.A. and Prescott, S.M. Leukotrienes C_4 and D_4 stimulate human endothelial cells to synthesize platelet-activating factor and bind neutrophils. **Proc. Natl. Acad. Sc. USA** 83 (1986) 2204-2208.
9. Jackson, R.J., Bolognese, B., Mangar, C.A., Hubbard, W.C., Marshall, L.A. and Winkler, J.D. The role of platelet-activating factor and other lipid mediators in inflammatory angiogenesis. **Biochim. Biophys. Acta** 1392 (1998) 145-152.
10. Kuliszkiewicz-Janus, M., Tuz, M.A. and Baczynski, S. Application of ^{31}P MRS to the analysis of phospholipid changes in plasma of patients with acute leukemia. **Biochim. Biophys. Acta** 1737 (2005) 11-15.
11. Tuz, M.A., Kuliszkiewicz-Janus, M. and Baczynski, S. Application of ^{31}P magnetic resonance spectroscopy to observation of phospholipid concentration changes in blood serum, plasma, peripheral blood mononuclear cells and bone marrow mononuclear cells from patients with hematological cancers – methodological review. **Polish J. Chem.** 80 (2006) 1009-1019.
12. Kuliszkiewicz-Janus, M., Tuz, M.A., Baczynski, S., Prajs, I. and Jaźwiec, B. ^{31}P MRS analysis of the phospholipid composition of normal human peripheral blood mononuclear cells (PBMC). **Cell. Mol. Biol. Lett.** 10 (2005) 373-382.
13. Folch, J., Lees, M. and Sloan-Stanley, G.H. A simple method for the isolation and purification of total lipids from animal tissues. **J. Biol. Chem.** 226 (1957) 497-509.
14. Bradamante, S., Barchiesi, E., Barenghi, L. and Zoppi, F. An alternative expeditious analysis of phospholipid composition in human blood plasma by ^{31}P NMR spectroscopy. **Anal. Biochem.** 185 (1990) 299-303.
15. Denizot, Y., Dupuis, F., Trimoreau, F., Praloran, V. and Liozon, E. Decreased levels of platelet-activating factor in blood of patients with lymphoid and non-lymphoid hematologic malignancies. **Blood** 85 (1995) 2992-2993.
16. Denizot, Y., Guglielmi, L., Donnard, M. and Trimoreau, F. Platelet-activating factor and normal or leukaemic haematopoiesis. **Leuk. Lymphoma** 44 (2003) 775-782.
17. Denizot, Y., Trimoreau, F., Dupuis, F., Verger, C. and Praloran, V. PAF and haematopoiesis: III. Presence and metabolism of platelet-activating factor in human bone marrow. **Biochim. Biophys. Acta** 1265 (1995) 55-60.
18. Dupuis, F., Rougier, F., Trimoreau, F., Ostyn, E., Dulery, C., Praloran, V. and Denizot, Y. Production and metabolism of platelet-activating factor by human bone marrow cells. **Res. Immunol.** 148 (1997) 119-126.

19. Donnard, M., Guglielmi, L., Turlure, P., Piguet, C., Couraud, M.J., Bordessoule, D. and Denizot, Y. Membrane and intracellular platelet-activating factor receptor expression in leukemic blasts of patients with acute myeloid and lymphoid leukemia. **Stem. Cells** 20 (2002) 394-104.
20. Berdel, W.E., Kulimova, E., Kolkmeyer, A., Zuhlsdorf, M., Serve, H., Buchner, T. and Oelmann, E. Receptor for platelet-activating factor (PAF) is not detectable by flow cytometry on the surface of myeloid leukemic cells. **Ann. Hematol.** 84 (2005) 771-773.
21. Garcia, M., Garcia, C., Gijon, M.A., Fernandez-Gallardo, S., Mollinedo, F. and Sanchez Crespo, M. Metabolism of platelet-activating factor in human haematopoietic cell lines. **Biochem. J.** 273 (1991) 573-578.
22. Trimoreau, F., Guglielmi, L., Touati, M., Faucher, J.L., Bordessoule, D. and Denizot, Y. Platelet-activating factor receptors on B cells of chronic lymphocytic leukaemia patients. **British J. Haematol.** 115 (2001) 711-712.
23. Guglielmi, L., Trimoreau, F., Donnard, M., Jaccard, A., Bordessoule, D. and Denizot, Y. Presence of membrane platelet-activating factor receptors on B cells of chronic B cell leukaemia patients. **Leuk. Lymphoma** 44 (2003) 1087-1088.
24. Foa, R., Bussolino, F., Ferrando, M.L., Guarini, A., Tetta, C., Mazzone, R., Gugliotta, L. and Camussi, G. Release of platelet-activating factor in human leukemia. **Cancer Res.** 45 (1985) 4483-4485.
25. Reynaud, S., Malissein, E., Donnard, M., Bordessoule, D., Turlure, P., Trimoreau, F. and Denizot, Y. Functional platelet-activating factor receptors in immature forms of leukemic blasts. **Leuk. Res.** 31 (2007) 399-402.