Vol. 98, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1981 Pages 349-358

CHANGES IN PHOSPHOLIPID AND FATTY ACID COMPOSITION IN DIFFERENTIATED FRIEND LEUKAEMIC CELLS

G. ZWINGELSTEIN[1], H. TAPIERO[2]§, J. PORTOUKALIAN[3] and A. FOURCADE[2]

[1] Groupe C.N.R.S. 33, Laboratoire de Physiopathologie Générale et Comparée, Université Claude-Bernard, 69622-Villeurbanne Cédex, France.

[2] Département de Pharmacologie Cellulaire et Moléculaire, I.N.S.E.R.M., Unité 50, I.C.I.G., 14-16, avenue Paul-Vaillant Couturier, 94800-Villejuif, France

[3] I.N.S.E.R.M., F.R.A. 24, Centre Léon Bérard, 69-Lyon, France.

Received October 29,1980

SUMMARY

Friend leukaemic cells (FLC) were induced to differentiate with dimethyl sulfoxyde (DMSO), hexamethylen-bis-acetamide (HMBA) and sodium butyrate (SB) and the phospholipid composition was analyzed. The phospholipid composition of differentiated cells differed from that of non differentiated cells and also varied according to inducer. The ratios of the percentage of phosphatidyl choline (PC) to that of phosphatidyl ethanolamine (PE) or sphingomyelin (SPH) increased by about 2-fold in DMSO or SB induced FLC. These ratios did not vary in HMBA induced FLC. Furthermore the fatty acid composition of PC and PE obtained from differentiated cells varied according to the inducer. Although these changes appeared to be related to the inducers, it can not be excluded that the differentiated state also contributes to these changes.

INTRODUCTION

Friend leukaemic cells (FLC) have been used as model for the study of erythroid differentiation (1). They

[2] § To whom request for reprints should be addressed.

0006-291X/81/020349-10$01.00/0

can be induced to differentiate and synthesize hemoglobin in vitro when grown in the presence of a variety of structurally unrelated agents (2 - 8). Among the agents particularly active as inducers are dimethyl sulfoxyde (DMSO), hexamethylen bis acetamide (HMBA) and sodium butyrate. Whether or not there is a common mechanism of action of the various agents is not yet established. However, it is known that the interaction of DMSO with phospholipid vesicles increases the phase transition temperature (9). Furthermore, DMSO and HMBA also alter the fluorescence polarization of FLC membranes (10), reduce membrane permeability for phosphate, uridine and leucine (11), increase agglutinability by lectins (12) and decrease the cell surface glycocalix (13). This suggests that their major effect may be on the cell membrane. They are few reports on changes of membrane components in differentiated cells. Recently changes in phospholipid composition have been shown in differentiated mouse myeloid leukaemia cells (14). The present study demonstrates changes in phospholipid and fatty acid composition of PC and PE and that these changes vary with the inducer.

MATERIALS AND METHODS

Cells. Friend leukaemic cells were derived from a clone of Friend virus transformed cells 745 A. Cells were grown in a modified Eagle's spinner medium lacking calcium and containing 10 mM sodium phosphate and non-essential aminoacids (Joklik modified Eagle's medium, GIBCO). Medium was supplemented with 10% fetal calf serum (GIBCO lot n°K 3862015) and antibiotics. All cell cultures were incubated at 37°C in a CO_2 incubator. Stimulation of globin synthesis was obtained by growing cells for 4 days in medium supplemented with 280 mM DMSO (dimethyl sulfoxyde, Sigma), or 4 mM hexamethylen bis acetamide (kindly donated by Y. Gazit) or 1 mM sodium butyrate (Merck). Benzidine reactive cells were determined as already described (10).

Lipid extraction and purification. Lipids were obtained from cells by several extractions with a chloroform-methanol mixture (1:1, v/v). The extract was taken to dryness, dissolved in chloroform-methanol (2:1, v/v) and filtered. The combined filtrates were subsequently washed once with 0.2 vol. of 0.9% KCl in water and once with 0.5 vol. of methanol-water (10:6, v/v) then the washed extract was evaporated to dryness under vacuum at 45°C and made up to a known volume of chloroform-ethanol (2:1, v/v).

Lipid analysis

Neutral lipids. Neutral lipids were identified after separation by thin layer chromatography, along with various known standards on silica gel G plates (Merck, Germany) developed with hexane-diethyl-ether-acetic acid (90:10:1, v/v). Free and esterified cholesterol were assayed according to Zlatkis et al. (15) after separation on thin layer chromatography.

Phospholipids. Phospholipids were separated by two-dimensional thin layer chromatography as already described (16). Quantification was measured by determining the phosphorus content (17) in each spot after staining with a specific spray reagent (18). Total lipid phosphorus was quantified by a modification of Bartlett(s method (17).

Gas chromatography of the fatty acid. Phosphatides (Phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine + phosphatidyl inositol) were purified on 0.5 mm thick layers silica gel H (Macherey Nagel, Dürer, Germany) prepared as already described (19), with chloroform-methanol-acetic acid water (60:30:7:3, by vol.) as developing solvent (20). After localization with primuline spray reagent under UV light (21), wet silica gel areas containing the phosphatides were rapidly scraped into dry screw-capped tubes, and transmethylated with methanolic-HCl reagent (22) was conducted according to a slight modification of method developed by Blough (23). After extraction of the fatty acid methylesters with petroleum ether (B.P. 50-60°C), gas chromatography was carried out isothermally at 190°C on a 0.22 cm i.d. and 3 m long stainless-steel column packed with 10% DEGS p, 80/100 mesh chromosorb W.A.W. The peaks were identified by comparison with standards. Quantitation was achieved on the major peaks by the planimetric method.

Protein determination. The protein was estimated on the delipidized residue obtained after lipid extraction. Aliquots of the delipidized residue were dissolved in NaOH, N. and the amount of protein was determined on a portion of this alkaline solution by Lowry's method (24).

RESULTS AND DISCUSSION

Friend leukaemic cells were induced to differentiate with DMSO, HMBA and sodium butyrate. The benzidine positive (B+) cells increased from 0 - 1% to 60 - 70% by the fourth day in the presence of each one of these inducers. In order to investigate changes of membrane components associated with the differentiated state of the cells, lipid, phospholipid and fatty acid composition of induced and non-induced FLC were analyzed. It is shown (table 1) that in differentiated cells, the phospholipid to cholesterol molar ratio increased and the amount of neutral lipid decreased. The extent of these changes depends on the inducer. HMBA appeared to be the most effective.

Table 1

THE EFFECT OF DIMETHYL SULFOXYDE (DMSO), HEXAMETHYLEN BIS ACETAMIDE (HMBA) AND SODIUM BUTYRATE ON THE LIPID CONTENT OF FRIEND LEUKAEMIC CELLS

	UNTREATED	DMSO	HMBA	BUTYRATE
TOTAL LIPIDS	27.1 ± 1.7	25.7	18.0 ± 1.0	22.3
PHOSPHOLIPIDS	9.01± 0.12	11.49 ± 0.24	9.7 ± 0.4	9.7 ± 0.17
TOTAL CHOLESTEROL	2.6 ± 0.2	2.8	2.2 ± 0.3	2.4
OTHER NEUTRAL LIPIDS	15.4 ± 1.3	11.5	6.2 ± 0.5	10.2
PHOSPHOLIPIDS TO CHOLESTEROL MOLAR RATIO §	1.66± 0.08	2.075± 0.06	2.32± 0.02	2.06± 0.11

Friend leukaemic cells were grown 4 days in medium supplemented or not with either DMSO (280mM), HMBA (4mM) or sodium butyrate (1mM). The lipid content of cells is determined by two separate experiments and the values reported are means of two different determinations of each experiment with range variations of 7-10 %. Values of lipid fractions are expressed as percent (w/w) of proteins and are given as mean ± S.D. when difference between treated and untreated cells is significant.

§A mean molecular weight of 825 is assumed for phospholipids.

The phospholipid composition of differentiated cells has been shown to change according to the inducer (table 2). The relative amount of phosphatidyl inositol and diphosphatidyl glycerol, (a phosphatide present only in cell mitochondria) increased in differentiated cells induced with HMBA whereas it decreased when induced with DMSO or sodium butyrate. The phosphatidyl choline to phosphatidyl ethanolamine and phosphatidyl choline to sphingomyelin ratios increased approximatively 2-fold in DMSO or sodium butyrate-induced FLC whereas they did not change after HMBA induction (table 2). Furthermore, the fatty acid composition of the two main phospholipids PC and PE from differentiated and undifferentiated FLC showed that changes were not related to the differentiated state of the cells. The fatty acid composition of PC from undifferentiated cells (table 3) showed that about 80 to 90% of the total fatty acids were represented by palmitic (16:0), palmitoleic (16:1) and oleic (18:1) acids. The polyunsaturated linoleic (18:2) and arachidonic (20:4) acids accounted for only 4-5%. In differentiated cells, changes occured according to the inducer. DMSO-induced cells were mainly characterized by a 2-fold increase of arachidonic acid and butyrate-induced cells by a decrease of oleic and linoleic acid. No significant changes were observed in HMBA-induced FLC. The fatty acid composition of PE purified from undifferentiated cells was different from that of PC. A major part (60-70%) was represented by palmitic (16:0), stearic (18:0) and oleic (18:1) acids. A minor, but important part (18-20%) was represented by the polyunsaturated arachidonic (20:4) and ercosapentaenoic (20:5) acids (table 4). Various changes occured in differentiated cells according to the inducer. Among these changes a 2-fold increase or arachidonic acid and about 2-fold decrease of linoleic acids in DMSO or butyrate induced FLC, whereas in HMBA induced cells only slight changes were obtained.

These results suggest, therefore, that changes in phospholipid composition and their fatty acids are determined by the inducer rather than the differentiated state of the cell. However, it is not excluded that the differentiated state contributes towards these changes. Studies on interactions of membrane components and particularly lipid-protein, may provide information on the role of membrane components in cellular differentiation.

Table 2

THE EFFECT OF DMSO, HMBA AND SODIUM BUTYRATE ON THE PHOSPHOLIPID COMPOSITION OF FRIEND LEUKAEMIC CELLS

PHOSPHOLIPID	UNTREATED	DMSO	HMBA	BUTYRATE
SPHINGOMYELIN (SPH)	4.6	2.8	4.1	3.3
PHOSPHATIDYL CHOLINE (PC)	50.9	61.7	46.7	66.8
PHOSPHATIDYL SERINE (PS)	3.0	4.5	5.8	4.1
PHOSPHATIDYL INOSITOL (PI)	8.2	6.5	10.2	6.4
PHOSPHATIDYL ETHANOLAMINE (PE)	25.2	17.5	22.7	14.1
DIPHOSPHATIDYL GLYCEROL	8.1	7.0	10.0	5.2
X (Unknown)			1.6	
PHOSPHATIDYL CHOLINE TO PHOSPHATIDYL ETHANOLAMINE MOLAR RATIO				
	2.02 $\pm$ 0.17	3.53	2.06 $\pm$ 0.02	4.74
PHOSPHATIDYL CHOLINE TO SPHINGOMYELIN MOLAR RATIO				
	10.31 $\pm$ 1.41	22.04 $\pm$ 0.93	11.39	20.24

Friend leukaemic cells were cultured for 4 days with or without 280 mM DMSO, 4 mM HMBA and 1 mM sodium butyrate. The cells were washed from times with NaCl 0.15 M, Tris 0.01 M pH 7.2. The last pellets are resuspended in 20 volumes of chloroform-methanol (2:1) and the analysis were carried out as described in Materials and Methods. (Results are expressed as percentage of the total lipid phosphorus).

Table 3

THE EFFECT OF DMSO, HMBA AND SODIUM BUTYRATE ON THE FATTY ACID COMPOSITION OF PHOSPHATIDYL CHOLINE PURIFIED FROM FRIEND LEUKAEMIC CELLS

FATTY ACID	UNTREATED	DMSO	HMBA	BUTYRATE
16: 0	32.5 ± 0.6	32.2	36.5 ± 3.0	44.9
16. 1	9.1 ± 0.3§	5.6 ± 0.3§	9.6 ± 0.5	9.0
17: ?	1.3 ± 0.2	1.2	1.7 ± 0.3	0.65
18: 0	1.5 ± 0.6	2.7	1.5 ± 0.4	2.1
18. 1	50.2 ± 1.1	50.5	45.4 ± 2.7	39.0
18: 2	2.3 ± 0.8	2.8	2.1 ± 0.4	1.2
20: 1	1.5 ± 0.1	1.7	1.6 ± 0.2	1.6
20: 4	1.0 ± 0.1§	2.6 ± 0.3§	1.4 ± 0.3	1.1
20: 5	0.3 ± 0.1	0.6	0.5 ± 0.1	0.5

Phosphatidylcholine of induced or not FLC was obtained as described in table 2. Fatty acid analysis was carried out as described in Materials and Methods. The results are expressed as weight % of total fatty acid. Polyunsaturated C22 fatty acid peaks and unidentified peaks less than 0.3% of total area are not evaluated.

§significant $p < 0.01$

Table 4

THE EFFECT OF DMSO, HMBA AND SODIUM BUTYRATE ON THE FATTY ACID COMPOSITION OF PHOSPHATIDYL ETHANOLAMINE PURIFIED FROM FRIEND LEUKAEMIC CELLS

FATTY ACID	UNTREATED	DMSO	HMBA	BUTYRATE
16: 0	11.8 ± 0.5§	8.0 ± 0.8§	14.2 ± 1.8	20.1
16: 1	3.8 ± 0.6§§	0.8 ± 0.5§§	4.6 ± 1.2	4.2 ± 0.7
17: ?	6.6 ± 2.2	11.5 ± 1.4	9.2 ± 1.7	2.2 ± 0.4
18: 0	10.3 ± 2.5	11.3 ± 0.1	8.6 ± 0.8	12.5 ± 2.2
18: 1	38.4 ± 0.4	34.7 ± 1.4	33.4 ± 0.5§	27.0
18: 2	7.9 ± 1.1§	2.5 ± 0.1§	4.8 ± 0.6	3.5 ± 0.6
20: 1	3.3 ± 1.1	1.2 ± 0.2	1.9 ± 0.5	1.6 ± 0.3
20: 4	13.3 ± 0.7§§	24.4 ± 0.5§§	17.9 ± 1.2§	24.6 ± 0.5
20: 5	4.7 ± 0.1	4.8 ± 0.5	5.6 ± 0.4	4.3 ± 0.8

Phosphatidyl ethanolamine of induced or not FLC was obtained as described in table 2. Fatty acid analysis was carried out as described in Materials and Methods. The results are expressed as weight % of total fatty acid. Polyunsaturated C22 fatty acid peaks and unidentified peaks less than 0.3% of total area are not evaluated.

§significant p 0.05, §§significant p 0.01

ACKNOWLEDGMENTS

We are indebted to Dr. J. Huppert for very helpful discussions. This study was supported by Contract D.G.R.S.T. 78.72.639 and 80.7.0445.

REFERENCES

1. Friend, C., Scher, W., Holland, J.G. and Sato, T. (1971) Proc. Natl. Acad. Sci. USA 68, 378-382.

2. Scher, W., Preisler, H.D. and Friend, C. (1973) J. Cell. Physiol. 81, 63-70.

3. Leder, A. and Leder, P. (1975) Cell 5, 319-322.

4. Gusella, J.F. and Housman, D. (1976) Cell 8, 263-269.

5. Ebert, P.S., Wars, I. and Buell, D.N. (1976) Cancer Res. 36, 1809-1813.

6. Bernstein, A., Hunt, D.M., Crichley, V. and Mak, T.W. (1976) Cell 9, 375-381.

7. Reuben, R.C., Wife, R.I., Breslow, R., Rifkind R.A. and Marks, P.A. (1976) Proc. Natl. Acad. Sci. USA 73, 862-866.

8. Ross, J. and Sautner, D. (1976) Cell 8, 513-520.

9. Lyman, G.H., Papahadjopoulos, D. and Preisler, H.D. (1976) Biochim. Biophys. Acta 448, 460-473.

10. Tapiero, H., Fourcade, A. and Billard, C. (1980) Cell. Diff. 9, 211-218.

11. Duke, S.K., Gaedicke, G., Kluge, N., Weinmann, B.J., Melderis, H., Steinheider, G., Crozier, T., Beckmann, H. and Ostertag W. (1974) Differentiation and control of malignancy of tumor cells. eds. Nakahara, W., Oho, T., Sugimura, T. and Sugano, H. (Tokyo University press) pp. 99-132.

12. Eisen, H., Nasi, S., Georgopoulos, C.P., Arnd-Jovin, D. and Ostertag, W. (1977) Cell 10, 685-689.

13. Sato, C., Kojima, K., Nishizawa, K. and Ikawa, Y. (1979) Cancer Res. 39, 1113-1117.

14. Honma, Y., Kasukabe, T. and Hozumi, M. (1980) Biochem. Biophys. Res. Comm. 93, 927-933.

15. Zlatkis, A., Zak, B. and Boyle, A.J. (1953) Lab. Clin. Med. 41, 486-498.

16. Portoukalian, J., Meister, R. and Zwingelstein, G. (1978) J. Chromatogr., 152, 569-574.

17. Bartlett, G.R. (1959) J. Biol. Chem., 234, 466-468.

18. Dittmer, J.C. and Lester, R.L. (1964) J. Lipid Res. 5, 126-131.

19. Zwingelstein, G., Abdul Malak, N. and Brichon, G. (1978) J. Thermal. Biol. 3, 229-233.

20. Skipski, V.P., Peterson, R.F. and Barclay, M. (1964) Biochem. J. 90, 374-381.

21. Skipski, V.P., Peterson, R.F., Sanders, J. and Barclay, M. (1963) J. Lipid Res. 4, 227-232.

22. Stoffel, W., Chu, F. and Arhens, E.M. (1959) Anal. Chem. 31, 307-315.

23. Blough, H.A., Weinstein, D.B., Lawson, D.E.M. and Kodicek, E. (1967) Virology 33, 459-466.

24. Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randal, R.J. (1951) J. Biol. Chem. 193, 265-275.