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THE EFFECT OF DIMETHYLSULFOXIDE ON THE LIPID COMPOSITION OF INDUCIBLE AND NON INDUCIBLE FRIEND LEUKEMIA CELLS

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Changes in phospholipids and in fatty acid composition were observed when Friend leukemia cells (FLC), but not the resistant one (RFLC) were exposed to DMSO. As a result of these changes, the molar ratio of phosphatidyl choline (PC) to phosphatidyl ethanolamine (PE) and the relative amount of arachidonic acid (20:4) isolated from total lipids, PC and PE increased in differentiated cells. We therefore conclude that erythroid differentiation induced by DMSO is related to changes in the lipid components of FLC membranes.

INTRODUCTION

The addition of certain dipolar aprotic solvents, such as DMSO, to growing murine erythroleukemia cells (FLC) induces a wide variety of erythrocyte markers (1 - 5). The initial biochemical event which signals the onset of differentiation is still unknown. However, various changes have been shown to occur in membrane properties. Among these changes, reduced membrane permeability to phosphate, uridine and leucine (6), increased agglutinability by lectins (7) and a decrease in the cell surface glycocalyx have been described (8). Considerable speculation has been directed towards the possible alteration

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of membrane properties during cell proliferation, differentiation, and malignant transformation (9). Moreover, the interaction between DMSO and phospholipid vesicles of FLC membranes suggested an alteration in lipid matrix properties (10). Recently, we reported that fluorescence polarization (P) of 1.6 diphenyl 1.3.5 hexatriene (DPH) embedded in FLC membrane varied according to the proliferating and differentiating state of the cell. In a cell variant resistant to DMSO induction, these P values were not affected (11 - 13).

The present study demonstrates specific changes in phospholipids and in fatty acid composition induced by DMSO in FLC, but not in RFLC.

MATERIALS AND METHODS

Cell culture. FLC 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. Cell densities were determined by cell count using a hemocytometer, and cell viability was measured by counting the cells excluding 0.1 % trypan blue. Stimulation of globin synthesis in the inducible FLC was obtained by growing cells for 4 to 6 days in medium supplemented with 280 mM DMSO (dimethyl sulfoxyde, Sigma). The benzidine reactive cells (B+ cells) were determined as previously described (12).

Isolation of a non inducible variant of FLC (RFLC). A variant of FLC (derived from lines 745 A) which is unable to synthesize hemoglobin in response to DMSO was isolated in our laboratory by seeding inducible cells at 0.5 x 10^6 cells/ml in medium supplemented with 280 mM DMSO (12). The cells were transferred every 5 days for three months, after which DMSO was removed and the cells, seeded at 0.1 x 10^6 cells/ml, were transferred every two days. These cells now have a low base level of B+ cells, ranging from 0 - 2 %, when grown in the presence of DMSO.

Lipid extraction and purification. Lipids were extracted from cells by several extractions with a chloroform-methanol mixture (1:1, v/v). The extract was dried, 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 evaporated to dryness under a vacuum at 45°C and made up to a known volume of chloroform-methanol (2:1, v/v).

Lipid analysis

Neutral lipids. They 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. (14) after separation on thin layer chromatography.
Phospholipids. They were separated by two-dimensional thin layer chromatography as already described (15). Quantification was done by determining the phosphorus content (16) in each spot after staining with a specific spray reagent (17). Total lipid phosphorus was quantified with a slight modification of Bartlett's method (16).

Gas chromatography of the fatty acid. Phosphatides (phosphatidyl choline, phosphatidyl ethanolamine and phosphatidyl serine + phosphatidyl inositol) were purified on 0.5 mm thick layers of silica gel H (Macherey Nagel, Dûrer, Germany) prepared as already described (18), with chloroform-methanol acetic acid water (60:30:7:3, by vol.) as the developing solvent (19). After localization with primuline spray reagent under UV light (20), wet silica gel areas containing the phosphatides were rapidly scraped into dry screw-capped tubes, and transmethylated with methanolic-HCl reagent (21) according to slight modification of the method developed by Blough (22). After extraction of the fatty acid methyl-esters 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 on 80/100 mesh chromosorb W.A.W. The peaks were identified by comparison with standards. Quantification 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 (23).

RESULTS

The addition of 280 mM DMSO to growing FLC leads to erythroid differentiation after an interval of 3 - 5 days. In previous studies using fluorescence polarization (P) with diphenyl hexatriene (DPH), we showed that changes in P values occurred during the differentiation process. In an FLC variant which is resistant to DMSO induction (RFLC), these changes in P values were not seen (12). In the present study, it was first asked whether erythroid differentiation induced by DMSO is associated with an alteration in cell membrane components. To investigate this question, phospholipids and fatty acid analysis were carried out in FLC and in RFLC exposed or not to DMSO. In FLC, the relative amount of lipids expressed as per cent (w/w) of proteins was 27.1 $\pm$ 0.8, among which were 9.01 $\pm$ 0.12 phospholipids, 2.6 $\pm$ 0.2 cholesterol and 15.4 $\pm$ 1.0 other neutral lipids. These relative amounts were similar in

Table 1 THE EFFECT OF DMSO ON PHOSPHOLIPID COMPOSITION OF FLC AND RFLC

	F L C		R F L C	
	UNTREATED	DMSO (280mM)	UNTREATED	DMSO (280mM)
SPHINGOMYELIN (SPH)	4.6 ± 0.2	2.8 ± 0.1§	4.8 ± 0.3	2.9 ± 0.3§§
PHOSPHATIDYL CHOLINE (PC)	50.9 ± 0.8	61.7 ± 1.2§	48.5 ± 0.6	47.9 ± 1.3
PHOSPHATIDYL SERINE (PS)	3.0 ± 0.1	4.5 ± 0.3§§	7.7 ± 0.7	7.2 ± 0.1
PHOSPHATIDYL INOSITOL (PI)	8.2 ± 0.2	6.5 ± 0.4§	10.7 ± 0.3	10.8 ± 0.5
PHOSPHATIDYL ETHANOLAMINE (PE)	25.2 ± 0.9	17.5 ± 0.5§	23.2 ± 0.3	22.2 ± 0.7
DIPHOSPHATIDYL GLYCEROL (DPG)	8.1 ± 0.2	7.0 ± 0.3	5.1 ± 0.6	8.5 ± 0.7
X				0.5 ± 0.2
MOLAR RATIOS				
PC : PE	2.02± 0.17	3.53± 0.21§§	2.09± 0.12	2.16± 0.22
PC : SPH	10.31± 1.41	22.08± 0.93§§	11.20± 0.97	17.51± 1.91
PI : PS	2.73± 0.22	1.44± 0.15	1.39± 0.15	1.5 ± 0.17

Friend leukemia cells (FLC) and a cell variant resistant to DMSO induction (RFLC) were cultured for five days in medium supplemented or not with DMSO (280mM). The phospholipid composition was determined in duplicate or triplicate from two separate experiments, as described in Materials and Methods. The values are expressed as per cent of total lipid phosphorus.

Statistical signification : (§) $p = 0.01$ (§§) $p = 0.05$

both variants, whether exposed or not to DMSO. Therefore, we investigated changes that might be involved in other lipid components.

The effect of DMSO on phospholipid composition

In order to study changes in membrane components associated with resistance to DMSO induction, phospholipids isolated from FLC and RFLC were analyzed. The phospholipid composition showed that the relative amounts of phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) and sphingomyeline (SPH) were similar in the two cells whereas those of phosphatidyl inositol (PI), phosphatidyl serine (PS) and phosphatidyl glycerol differed significantly. As a result of these changes, the molar ratios of PI to PS were lower in RFLC than in FLC (table 1). If the DMSO effect which induces erythroid differentiation in FLC is related to changes in cell membrane components, one might expect to obtain these changes in the inducible, but not in the non inducible, cell variant. The data in table 1 confirm this expectation. When FLC were exposed for 5 days to 280 mM DMSO, about 85 % of the cells were induced to differentiate. The phospholipid composition of these cells showed an increase in the relative amount of PC and PS, and a decrease in SPH, PI, PE and diphosphatidyl glycerol. As a result of these changes, the molar ratio of PC to PE and PC to SPH increased, respectively by 75 % and 114 %, whereas the molar ratio of PI to PS decreased by about 90 %. The effect of DMSO on the phospholipid composition of RFLC was different, as expected. The relative amounts of PC, PS, PI and PE remained unchanged compared to untreated cells, whereas those of SPH decreased under DMSO treatment, just as in the inducible cell variant. Since the degree of saturation of the phospholipid-acyl chains can also interfere with membrane fluidity (24, 25), the fatty acid composition of total lipids, phosphatidyl choline and phosphatidyl ethanolamine was analyzed.

The effect of DMSO on fatty acid composition

The fatty acid analysis of total lipids (table 2) and phosphatidyl choline (table 3) isolated from both cell variants showed that more than 90 % of fatty acids are represented by palmitic (16:0), palmitoleic (16:1), stearic (18:0) and oleic acids (18:1). The relative amount of polyunsaturated linoleic

Table 2 THE EFFECT OF DMSO ON FATTY ACID COMPOSITION OF LIPIDS EXTRACTED FROM FLC AND RFLC

FATTY ACID	F L C		R F L C	
	UNTREATED	DMSO (280mM)	UNTREATED	DMSO (280mM)
16 : 0	39.1 ± 0.2	39.7 ± 0.3	36.7 ± 0.8	43.1 ± 1.3§§
16 : 1	9.2 ± 0.4	5.3 ± 0.6§§	10.3 ± 0.5	8.3 ± 0.7
18 : 0	11.2 ± 0.1	11.1 ± 0.3	13.7 ± 0.4	13.6 ± 0.1
18 : 1	34.0 ± 0.6	32.5 ± 0.5	30.9 ± 0.8	30.7 ± 0.9
18 : 2	3.3 ± 0.2	3.5 ± 0.2	4.7 ± 0.4	2.1 ± 0.8
20 : 4	3.2 ± 0.2	7.8 ± 0.5§	3.7 ± 0.4	2.2 ± 0.6

Friend leukemia cells (FLC) and a cell variant resistant to DMSO induction (RFLC) were cultured for five days in medium supplemented or not with 290mM DMSO. Total lipids were extracted and fatty acid analysis was carried out in duplicate or triplicate from two separate experiments, as described in Materials and Methods. The values are expressed as weight per cent of total fatty acids.

Statistical signification : (§) $p = 0.01$ (§§) $p = 0.05$

Table 3 THE EFFECT OF DMSO ON FATTY ACID COMPOSITION OF PHOSPHATIDYL CHOLINE ISOLATED FROM FLC AND RFLC

FATTY ACID	F L C		R F L C	
	UNTREATED	DMSO (280mM)	UNTREATED	DMSO (280mM)
16 : 0	32.9 ± 0.6	32.6 ± 0.8	31.2 ± 0.7	36.7 ± 1.3
16 : 1	9.2 ± 0.3	5.7 ± 0.3§	7.5 ± 0.6	10.0 ± 0.9
18 : 0	1.5 ± 0.6	2.7 ± 0.7	3.9 ± 0.4	3.4 ± 0.7
18 : 1	50.9 ± 1.1	51.1 ± 0.9	50.7 ± 1.3	43.2 ± 1.4
18 : 2	2.3 ± 0.8	2.8 ± 0.6	3.9 ± 0.7	3.3 ± 0.6
20 : 1	1.5 ± 0.1	1.7 ± 0.4	2.0 ± 0.1	1.4 ± 0.3
20 : 4	1.0 ± 0.1	2.6 ± 0.3§§	1.0 ± 0.1	1.5 ± 0.4
20 : 5	0.3 ± 0.1	0.6 ± 0.2	0.3 ± 0.1	0.7 ± 0.2

Fatty acid analysis of phosphatidyl choline isolated from FLC and RFLC exposed or not to 280mM DMSO (see table 1) was carried out in duplicate or triplicate from two separate experiments, as described in Materials and Methods. The values are expressed as weight per cent of the total fatty acids.

Statistical signification : (§) $p = 0.01$ (§§) $p = 0.05$

Polyunsaturated C_{22} fatty acid peaks and unidentified peaks less than 0.3 % of total area were not evaluated.

Table 4 THE EFFECT OF DMSO ON FATTY ACID COMPOSITION OF PHOSPHATIDYL ETHANOLAMINE ISOLATED FROM FLC AND RFLC

FATTY ACID	F L C		R F L C	
	UNTREATED	DMSO (280mM)	UNTREATED	DMSO (280mM)
16 : 0	12.6 ± 0.5	9.0 ± 0.7§§	19.6 ± 1.1	16.3 ± 0.9
16 : 1	4.1 ± 0.4	0.9 ± 0.5§§	5.6 ± 0.7	5.7 ± 0.6
18 : 0	11.0 ± 2.7	12.8 ± 0.1	14.8 ± 1.1	12.0 ± 0.9
18 : 1	41.1 ± 0.4	39.2 ± 1.6	35.9 ± 1.1	31.7 ± 1.2
18 : 2	8.5 ± 1.2	2.8 ± 0.1§§	2.1 ± 0.9	3.9 ± 0.8
20 : 1	3.5 ± 1.2	1.4 ± 0.2	1.9 ± 0.6	1.6 ± 0.4
20 : 4	14.2 ± 0.8	27.6 ± 0.6§	15.2 ± 1.0	15.4 ± 0.9
20 : 5	5.0 ± 0.1	5.4 ± 0.6	4.7 ± 0.2	5.2 ± 0.3

Fatty acids analysis of phosphatidyl ethanolamine isolated from FLC and RFLC exposed or not to 280mM DMSO (see table 1) was carried out in duplicate or triplicate from two separate experiments,as described in Materials and Methods The values are expressed as weight per cent of total fatty acids.

Statistical signification : (§) $p = 0.01$ (§§) $p = 0.05$

(18:2), arachidonic (20:4) and eicosapentaenoic acids (20:5) corresponded to a minor fraction. As compared to phosphatidyl choline, the fatty acid analysis of phosphatidyl ethanolamine showed a lower amount in palmitic acid (16:0) and a higher amount in polyunsaturated fatty acids. The relative amount of arachidonic acid (20:4) accounted for about 15 % in both cell variants (table 4). When erythroid differentiation was induced by DMSO, the fatty acid composition of total lipids, phosphatidyl choline and phosphatidyl ethanolamine was modified. The relative amount of arachidonic acid (20:4) increased by 143 %, 160 % and 94 % in total lipids, phosphatidyl choline and phosphatidyl ethanolamine, respectively. In RFLC treated with 280 mM DMSO, the relative amount of arachidonic acid isolated from these fractions did not change. We therefore conclude that in FLC, erythroid differentiation induced by DMSO is associated with changes in the metabolic pathway of the lipids. These changes are characterized by an increase in the PC to PE molar ratio and a modification in their fatty acid composition.

DISCUSSION

The purpose of this study was to investigate whether a relationship exists between the lipid composition and the differentiated state of Friend leukemia cells (FLC). In this study a significant decrease of SPH and PE occured after five days of FLC exposure to DMSO when the population contained the maximal number of differentiated cells. As a result of these changes the molar ratio of PC to PE and PC to SPH increased, respectively by 75 % and 114 %. These findings are in contrast to those reported by others who describe that as a result of FLC maturation a relative constant level (26) or an increase (27) of PE were observed. The mechanism by which the phospholipid composition changes is still unclear. A decrease of PE synthesis in DMSO exposed FLC was reported to be an early effect (26). It can also be suggested that DMSO induces a transient alteration in the phospholipid metabolic pathway, resulting in a modification of the PC to PE molar ratio. Studies with erythroid cells have shown that two membrane bound enzymes can synthesize PC by successive N methylation of PE (28). This pathway which is stimulated by isoproterenol is inhibited by d,l-propanolol, a β adrenergic antagonist (29) and also an inhibitor of DMSO induced FLC (30). Moreover it was recently shown that β adrenergic receptors increase in differentiated FLC membranes induced by DMSO (31) and in reticulocyte ghosts, through stimulation of phospholipid methylation. The increase in the relative amount of arachidonic acid (20:4) of the two main phospholipids PC and PE was also observed in differentiated FLC (32) and reported also in mastocytoma P815 cells treated by other compounds, such as sodium butyrate (33). The mechanism by which this increase occurs is still unknown. Althouth arachidonic acid is known as a precursor of various compounds which act on cell growth and FLC differentiation (34) the events to the process of cell differentiation is unclear. Because changes in phospholipid and fatty acids composition are prevalent in sensitive but not in resistant FLC, a casual relationship may be inferred. However the exact role of these metabolic changes is yet to be defined.

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