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SELF-ADAPTIVE MODIFICATION OF RED-CELL MEMBRANE LIPIDS IN LECITHIN: CHOLESTEROL ACYLTRANSFERASE DEFICIENCY

LIPID ANALYSIS AND SPIN LABELING

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In a patient with lecithin: cholesterol acyltransferase deficiency, free cholesterol was markedly increased, and esterified cholesterol was diminished. In the patient's plasma, an increase in phosphatidylcholine (PC) and a decrease in sphingomyelin were observed. Concomitantly, an increase in a shorter acyl chain 16:0 was noted in PC, sphingomyelin and phosphatidylethanolamine (PE). In contrast to these results, longer chains such as 22:0 and 24:0 were decreased, especially in sphingomyelin. Unsaturated double bonds such as 18:1 was also increased in PC and PE. In the red-cell membrane lipids, the increase in free cholesterol was counteracted by an increase in PC and by a decrease in sphingomyelin and PE, reflecting changes in the patient's plasma lipids. Increased 16:0 (in PC) and decreased 18:0 and 24:0 were observed. The increased plasma free cholesterol due to metabolic defect (lecithin: cholesterol acyltransferase deficiency) led to decreased red-cell membrane fluidity. This effect appeared to be counteracted by changing phospholipid composition (increased PC and decreased sphingomyelin and PE), by increasing shorter chains (16:0), by decreasing longer chains (18:0 and 24:0) and by increasing unsaturated double bonds (18:2). These results can be interpreted as a self-adaptive modification of lecithin: cholesterol acyltransferase deficiency-induced red-cell membrane abnormalities, to maintain normal membrane fluidity. This speculation was supported by the ESR spin-label studies on the patient's membrane lipids. The normal order parameters in intact red cells and in total lipid liposomes were decreased if cholesterol-depleted membrane liposomes were prepared. Thus, the hardening effect of cholesterol appeared to be counteracted by the softening effects described above. Overall membrane fluidity in intact red cells of the lecithin: cholesterol acyltransferase-deficient patient was maintained normally, judged by order parameters in ESR spin-label studies.

Introduction

Red-cell membrane lipid abnormalities [1] of congenital or acquired origin have recently been described, such as hereditary high red-cell membrane phosphatidylcholine hemolytic anemia [2,3], abetalipoproteinemia [4], spur cell anemia [5] and liver disease with target cells [6]. Deficiency of the plasma enzyme, lecithin : cholesterol acyltransferase is known to produce marked alteration in plasma lipids, especially increased free cholesterol [7]. The membrane lipids in mature red cells were

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Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; 5-SAL, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl.

dependent on the plasma lipids because of the lack of de novo synthesis of the membrane lipids [8]. Thus, the marked abnormality of plasma lipids in lecithin : cholesterol acyltransferase deficiency should affect the red-cell membrane lipids [9]. However, it is also shown that the increase in red-cell membrane fluidity in lecithin : cholesterol acyltransferase deficiency is surprisingly small in view of the extensive alterations both in membrane lipid composition and in the functional properties of these cells [9]. Free cholesterol is known as a hardening effector in red-cell membrane fluidity, and the decreased membrane fluidity may lead the abnormal red cells to early destruction, through, for instance, increased hemolysis. Thus, the compensation mechanism against increased free cholesterol is interesting to investigate both in the plasma and in the red cells in cases of lecithin : cholesterol acyltransferase deficiency. For this purpose, the classes of lipid, the fatty acid composition, the length of the chains and the saturation of the double bonds were analyzed in the plasma lipids and the red-cell membrane lipids. Finally, the membrane fluidity of the patient's red cells was determined in the intact red cells, in the liposomes of total lipids and in cholesterol-depleted liposomes. The self-adaptive modification mechanism in these lecithin : cholesterol acyltransferase deficiency red cells with marked membrane lipid abnormalities will be discussed.

Materials and Methods

Extraction and analysis of lipids

Freshly drawn heparinized venous blood was centrifuged at $250 \times g$ for 10 min to separate red cells from the plasma [10]. The red cells were washed three times in isotonic saline (pH 7.4) by centrifugation, and the hematocrit was adjusted to 30%. The plasma was further centrifuged at 3000 $\times g$ for 10 min to eliminate remaining white cells and platelets.

Extraction of lipids from the plasma was performed by the method of Folch et al. [11]. Red-cell lipids were extracted using isopropyl alcohol and chloroform according to the method of Broekhuyse [12]. Phospholipid phosphorus was determined by the method of Bartlett [13]. Phospholipids were separated by thin-layer chromatography on Silica-Gel H (Merck) developed with the solvent system chloroform/ methanol/ acetic acid/ water (30 : 15 : 4 : 2, v/v). The determination of individual phospholipids was carried out according to the method of Siakotos and Rouser [14]. For the analysis of the fatty acid composition of individual phospholipids, the corresponding spots on thin-layer chromatography plates were scraped. Fatty acids of the phospholipids were converted to methyl esters by byron trifluoride treatment [15], and examined with a Gas Chromatograph GC-6A (Shimadzu, Japan) equipped with a digital integrator, Chromatopac E1A. Gas chromatography was carried out using a glass column (200 cm $\times$ 3 mm) packed with 10% diethylene glycol succinate polyester.

Spin-labeling studies of intact red cells and extracted lipids

Spin-labeling studies were performed on liposome of extracted total lipids from the red cells, and on cholesterol-depleted phospholipid liposomes of the red cells, and also on intact red cells.

A stearic acid spin probe, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl (5-SAL), was purchased from Syva Co. and utilized without further purification. For preparation of spin-labeled lipid dispersions, special care was taken to prevent the denaturation of lipids by using butylated hydroxytoluene (5 μg/ml) as an antioxidant under a nitrogen stream. Extracted lipids were mixed with 1 mol% of 5-SAL in a test-tube, and the solvent was evaporated under nitrogen gas. 200 μl of 50 mM Tris-buffered saline (pH 7.5) was added into the test-tube with a few glass beads and vortexed with a thermomixer for 1 min at 40°C [16]. The spin-labeled lipid dispersions were taken into a glass capillary, and ESR spectra were recorded on a JEOL IX ESR Spectrometer at various temperatures. The determinations were performed in triplicate. The order parameter, S, an index of fluidity, was calculated using the following formula:

$$S = \frac{T'_{\parallel} - T'}{T_{zz} - \frac{1}{2}(T_{xx} + T_{yy})} \cdot \frac{a}{a'}$$

where T_{zz}, T_{xx} and T_{Yy} are the hyperfine principal values of the nitroxide radical [16] and a/a' is a

correction coefficient for the change of polarity in an aqueous solution [17].

The order parameter measures the mean value of $(3\cos^2\theta - 1)/2$ where θ is the angle between one of the principal axes of the nitroxide moiety and the axis of the axial symmetry. The smaller S becomes, the greater the spread in θ or the amplitude of rapid anisotropic motion. In the present paper, we use the order parameter of the fatty acid spin label incorporated into the membrane for discussion of the fluidity.

Results

The patient (49-year-old male) was found to have compensated hemolysis, hepatosplenomegaly without liver dysfunctions, and a marked poikilocytosis in red-cell morphology. The activities of plasma enzyme, lecithin : cholesterol acyltransferase (nmoles/ml per h) in the patient were markedly deficient in determinations both by the method of Glomset and Wright (9.6 ± 11.1, $n = 20$: control 93.3 ± 16.8, $n = 20$) and by that of Stokke and Norum (2.3 ± 1.4, $n = 3$: control 66.3 ± 11.1, $n = 20$) [7]. The red-cell membrane studies revealed normal composition of membrane proteins on sodium dodecyl sulfate polyacrylamide gel disc electrophoresis [18], and decreased sodium influx (0.93 mmol/liter erythrocytes per h, $n = 4$: control 1.49 ± 0.14, $n = 54$) [7].

Plasma lipid composition

Free cholesterol was markedly elevated in the patient's plasma, contrary to the significant decrease of the esterified form due to the deficiency of lecithin : cholesterol acyltransferase activity as shown in Table I. High-density lipoprotein (HDL) cholesterol was also diminished remarkably. Among plasma phospholipids, sphingomyelin was decreased ($P < 0.01$), and PC was slightly increased. Lipoprotein X was detected on agar gel electrophoresis, in addition to an increase in triacylglycerols.

Red-cell membrane lipid composition

A marked abnormality was noted in red-cell membrane lipids of the patient, especially the elevation of free cholesterol ($P < 0.01$), associated with an profound increase in PC ($P < 0.01$), as shown in Table II. In contrast, the level of

TABLE I

PLASMA LIPIDS IN A PATIENT WITH CONGENITAL LECITHIN : CHOLESTEROL ACYLTRANSFERASE DEFICIENCY

	Patient ($n = 4$)	Normal ($n = 5$)
Cholesterol (mg/dl)		
Total	194 ±28	178 ±32
Free	176 ±26 (90.6±1.3%) [a]	49 ± 9 (27.5± 5.0%)
Esterified	18 ± 3 (9.4±0.2%) [a]	129 ±23 (72.5±12.9%)
High-density-lipoprotein cholesterol (mg/dl)	3 ± 1 [a]	50 ±11
Phospholipids (%)		
Lysophosphatidylcholine	0.1± 0.1	4.8± 2.4
Phosphatidylcholine	77.9± 4.8	69.2± 6.3
Sphingomyelin	8.4± 1.2 [b]	17.2± 2.7
Phosphatidylethanolamine	7.1± 2.1	3.4± 3.1
Phosphatidylserine + phosphatidylinositol	5.0± 2.1	2.3± 2.3
Others	1.5± 0.9	2.2± 2.6
Triacylglycerols (mg/dl)	284 ±39 [b]	95 ±65
Lipoprotein X	present	–

Significant in $P < 0.001$ (a) and in $P < 0.01$ (b).

TABLE II

RED CELL MEMBRANE LIPIDS IN A PATIENT WITH CONGENITAL LECITHIN:CHOLESTEROL ACYLTRANSFERASE DEFICIENCY

Values $\mu g/10^{10}$ red blood cells. Numbers in parentheses represent percentage of phospholipids.

	Patient ($n = 3$)	Normal ($n = 5$)
Free cholesterol (FC)	1763 [a]	1202 ±103
Total phospholipids (PL)	3435 [a]	2604 ±241
Lysophosphatidylcholine (L-PC)	21 (0.6)	34 ± 18 (1.3±0.7)
Phosphatidylcholine (PC)	1738 (50.6) [a]	747 ± 73 (28.7±2.8)
Sphingomyelin (SM)	546 (15.9) [a]	674 ± 49 (25.9±1.9)
Phosphatidylethanolamine (PE)	687 (20.0) [a]	805 ± 42 (30.9±1.6)
Phosphatidylserine (PS) + phosphatidylinositol (PE)	443 (12.9)	344 ± 34 (13.2±1.3)
(PC+SM+L-PC)/(PE+PS+PI)	2.04	1.27± 0.04
FC/PL ratio	1.00	0.90± 0.04

[a] Significant in $P < 0.01$.

TABLE III

FATTY ACID COMPOSITION OF PLASMA LIPIDS OF A PATIENT WITH CONGENITAL LECITHIN:CHOLESTEROL ACYLTRANSFERASE DEFICIENCY

Values are percentages. Triplicate determinations. DMA, dimethylacetal derivatives.

	Total lipids		Phosphatidylcholine (PC)		Sphingomyelin (SM)		Phosphatidylethanolamine (PE)	
	Lecithin: cholesterol acyltransferase deficiency ($n = 3$)	Normal ($n = 5$)	Lecithin: cholesterol acyltransferase deficiency ($n = 3$)	Normal ($n = 5$)	Lecithin: cholesterol acyltransferase deficiency ($n = 3$)	Normal ($n = 5$)	Lecithin: cholesterol acyltransferase deficiency ($n = 3$)	Normal ($n = 5$)
DMA	–	–	–	–	–	–	2.9	5.7±1.1
16:0	25.9 [a]	21.0±1.1	34.0 [a]	28.9±1.1	37.4 [a]	25.4±0.4	17.4 [a]	14.4±1.4
16:1	2.4	1.6±0.9	–	–	–	–	–	–
DMA	–	–	–	–	–	–	3.3	5.7±0.7
18:0	9.9	7.5±0.4	12.8 [a]	14.5±0.3	8.5	7.7±0.7	17.4 [a]	13.9±1.2
18:1	29.2 [a]	18.6±2.3	13.9	10.5±0.5	5.4	4.3±1.3	17.0 [a]	10.2±1.4
18:2	18.2 [a]	33.5±4.5	26.1	26.1±2.4	6.1	6.3±2.3	15.8 [a]	19.0±3.2
20:0	–	–	–	–	2.8 [a]	4.1±0.6	–	–
20:1	–	–	–	–	–	–	–	–
20:3	0.4	1.0±0.3	1.0	2.2±0.5	–	–	–	–
20:4	3.1 [a]	5.7±0.6	4.1 [a]	7.1±0.7	–	–	9.4	11.2±1.7
20:5	1.8	1.7±0.6	2.7	2.4±0.6	–	–	4.6 [a]	2.4±0.7
22:0	–	–	–	–	6.2 [a]	11.3±2.2	–	–
22:1	–	–	–	–	1.3	1.7±0.5	–	–
22:2	–	–	–	–	2.4	4.7±0.8	–	–
22:5	–	–	–	–	1.2	1.9±0.4	0.9	1.1±0.3
22:6	3.2	4.0±0.7	3.8 [a]	5.3±0.5	–	–	9.8	9.7±1.7
24:0	0.3	0.5±0.1	–	–	4.2 [a]	7.9±1.0	–	–
24:1	0.6	0.9±0.1	–	–	21.7	19.9±2.6	–	–

[a] Significant in $P < 0.01$.

TABLE IV

FATTY ACID COMPOSITION OF RED-CELL LIPIDS OF A PATIENT WITH CONGENITAL LECITHIN:CHOLESTEROL ACYL TRANSFERASE DEFICIENCY

Values are percentages. DMA, dimethylacetal derivatives.

	Total lipids		Phosphatidylcholine		Sphingomyelin		Phosphatidyl-ethanolamine		Phosphatidylserine + phosphatidylinositol	
	Lecithin: cholesterol acyltransferase deficiency ($n = 3$)	Normal ($n = 5$)	Lecithin: cholesterol acyltransferase deficiency ($n = 3$)	Normal ($n = 5$)	Lecithin: cholesterol acyltransferase deficiency ($n = 3$)	Normal ($n = 5$)	Lecithin: cholesterol acyltransferase deficiency ($n = 3$)	Normal ($n = 5$)	Lecithin: cholesterol acyltransferase deficiency ($n = 3$)	Normal ($n = 5$)
DMA	–	–	–	–	–	–	6.0 [a]	4.8 ± 0.3	–	–
16:0	26.8 [a]	21.8 ± 1.2	41.7 [a]	34.9 ± 0.8	24.1 [b]	26.2 ± 1.1	15.3	16.0 ± 0.5	2.8	3.8 ± 0.7
DMA	–	–	–	–	–	–	9.1	8.0 ± 0.9	–	–
18:0	12.5 [a]	14.3 ± 0.4	7.7 [a]	12.2 ± 0.4	5.7 [a]	7.1 ± 0.5	9.9 [a]	8.7 ± 0.2	48.4	46.7 ± 2.8
18:1	11.2 [a]	13.4 ± 1.0	14.0 [a]	17.2 ± 0.6	4.2 [a]	2.4 ± 0.6	11.3 [a]	17.0 ± 0.8	4.8 [a]	8.5 ± 0.7
18:2	15.3 [a]	10.8 ± 1.0	24.3 [b]	22.6 ± 1.0	4.8 [a]	2.2 ± 0.8	5.8	6.6 ± 1.2	2.4	2.3 ± 0.2
20:4	11.1	12.0 ± 0.8	4.8	4.6 ± 0.6	–	–	17.1	16.4 ± 0.9	15.5 [a]	18.2 ± 0.7
20:5	3.1 [a]	1.5 ± 0.5	2.8 [a]	1.6 ± 0.4	–	–	5.5 [a]	3.0 ± 0.7	1.1	0.9 ± 0.4
22:0	–	–	–	–	6.0 [a]	7.0 ± 0.4	–	–	–	–
22:4	–	–	–	–	–	–	2.8	3.4 ± 0.5	1.9	2.0 ± 0.3
22:5	2.2 [b]	2.8 ± 0.3	–	–	2.7 [a]	4.4 ± 0.5	4.1	3.8 ± 0.4	4.3 [a]	3.2 ± 0.4
22:6	7.6	7.2 ± 0.9	3.2	2.9 ± 0.4	–	–	11.7 [a]	9.5 ± 0.9	16.1 [a]	10.8 ± 0.9
24:0	2.7 [a]	4.9 ± 0.7	–	–	21.3	19.1 ± 0.8	–	–	–	–
24:1	2.5 [a]	4.4 ± 0.9	–	–	26.3	26.6 ± 1.5	–	–	–	–

Significant in $P < 0.01$ (a) and in $P < 0.02$ (b).

sphingomyelin and the PE content were significantly decreased ($P < 0.01$). A ratio of free cholesterol to total phospholipids was maintained normally, in spite of these marked abnormalities of red-cell membrane lipids. Increased free cholesterol in the patient's red cells was counteracted chiefly by the increased PC.

Fatty acid composition of plasma lipids

In the fatty acid composition of total lipids, a shorter-chain 16 : 0 was increased ($P < 0.01$), and unsaturated fatty acids, such as 18 : 1 and 18 : 2, were also increased ($P < 0.01$) as shown in Table III.

For PC extracted from the patient's plasma, an increase in a shorter-chain 16 : 0 and the decrease in longer-chain 18 : 0 were observed ($P < 0.01$). In the case of sphingomyelin, 16 : 0 was increased ($P < 0.01$), concomitant with a decrease in 22 : 0 and 24 : 0 ($P < 0.01$). 16 : 0 was also increased in PE.

Fatty acid composition of red-cell lipids

In the case of total lipids, a shorter-chain 16 : 0 was increased, and longer chains 18 : 0 and 24 : 0 were somewhat decreased, as shown in Table IV. Unsaturated fatty acids, such as 18 : 2 and 20 : 5, were increased ($P < 0.01$). The overall unsaturation index (160.8: control 152.1) was significantly increased ($P < 0.01$).

In PC, 16 : 0 was also increased, contrary to the decrease 18 : 0 as a longer acyl chain. The unsaturation index (115.0: control 106.2) was also increased ($P < 0.01$).

In the cases of PE, sphingomyelin and phosphatidylserine + phosphatidylinositol, no major changes were observed, except for a slight increase of polyunsaturated chains in the PE and phosphatidylserine fractions.

Analysis of ESR spectra of spin-labeled phospholipids extracted from the red cells of the patient

For the liposomes of total lipids, the liposomes free of cholesterol prepared from the red-cell membranes, and intact red cells of normal subjects and of the patient, the parameter, S, was calculated from results of the ESR spectra. Generally, the increased free cholesterol content, as observed in the patient, decreases membrane fluidity, whereas the concomitantly increased PC content enhances red-cell membrane fluidity.

In the phospholipid liposomes free of cholesterol, order parameters in ESR were decreased over the lower temperature range, as shown in Fig. 1. In contrast to these results, total lipid liposomes, including cholesterol, showed no abnormalities over a wide range of temperature. Thus, the cholesterol-depleted phospholipid liposomes appear to possess increased fluidity.

For the intact red cells, the order parameters of ESR spectra in the patient were normal at various temperatures (Table V), as with the results for the total lipids with enhanced cholesterol. Thus, membrane proteins appeared not to affect the order parameters in ESR spectra.

TABLE V

ORDER PARAMETERS IN ESR FOR INTACT RED CELLS OF A PATIENT WITH CONGENITAL LECITHIN : CHOLESTEROL ACYLTRANSFERASE DEFICIENCY

Determinations in triplicate

	Temperature (°C)	$2T$ (Parallel) (G)	$2T$ (perpendicular) (G)	a'(G)	S
Lecithin:	47	52.5	19.0	15.1	0.613
cholesterol	45	52.8	18.8	15.1	0.623
acyltransferase	43	53.0	18.6	15.0	0.631
deficiency	41	53.4	18.5	15.1	0.639
	39	53.8	18.4	15.1	0.647
	37	54.1	18.1	15.1	0.660
	35	54.3	18.2	15.1	0.659
	33	54.9	18.0	15.2	0.672
	31	55.5	17.9	15.2	0.682
	29	56.2	17.7	15.3	0.696
	27	56.5	17.6	15.3	0.702
Control	47	52.6	18.8	15.0	0.620
	45	52.9	18.7	15.1	0.627
	43	53.6	18.5	15.1	0.641
	41	53.8	18.4	15.1	0.647
	39	54.1	18.3	15.1	0.653
	37	54.1	18.2	15.1	0.657
	35	54.8	18.1	15.2	0.668
	33	55.4	17.9	15.2	0.681
	31	56.0	17.7	15.2	0.694
	29	56.3	17.6	15.3	0.700
	27	57.4	17.5	15.4	0.715
	25	57.7	17.3	15.4	0.725

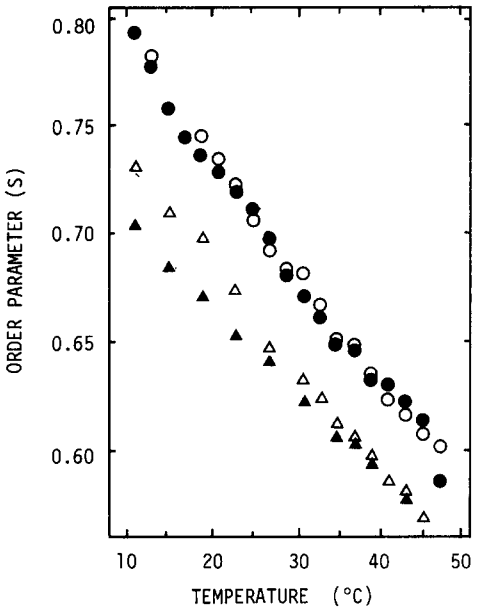

Fig. 1. ESR spin-labeling studies on the liposomes prepared from the red cells of a patient with lecithin:cholesterol acyltransferase deficiency. Open circles denote results on the total lipid liposomes of normal red cells, and closed circles those of lecithin:cholesterol acyltransferase-deficient red cells, respectively. Open triangles depict results on the free cholesterol-depleted phospholipid liposomes of normal red cells, and closed triangles those of lecithin:cholesterol acyltransferase-deficient red cells, respectively.

Discussion

The membrane fluidity is known to be dependent on the type of cholesterol (free or esterified), the classes of phospholipid, the molar ratio of cholesterol to phospholipids, the saturation of the double bonds, the number of carbons in the acyl chains, and the presence or absence of amphipathic compounds such as lysophosphatides [1,19–22].

Free cholesterol decreases the membrane lipid fluidity, while esterified cholesterol increases it. PC increases membrane fluidity, and sphingomyelin and PE decreases it. The shorter acyl chain length and the increased unsaturated fatty acids of these phospholipids enhances the membrane fluidity, in contrast to the decrease in membrane lipid fluidity by the presence of the longer acyl chain length and by increasing the saturation of fatty acids. Lysophosphatides are known to increase the fluidity.

The results in this communication can be summarized as shown in Fig. 2.

Due to the deficiency of the plasma enzyme, lecithin:cholesterol acyltransferase, free cholesterol accumulated in the patient's plasma. Thus, the increased free cholesterol decreases the membrane lipid fluidity substantially.

It is interesting enough to note that, counteracting the higher concentration of free cholesterol, PC was markedly increased, which may ease the decreased fluidity caused by free cholesterol. Sphingomyelin in the patient's plasma was decreased which will also alleviate the decreased fluidity. Shorter carbon acyl chains (such as 16:0) were increased in total plasma lipids, PC, sphingomyelin and PE, and longer carbon ones were decreased (such as 22:0 and 24:0 in sphingomyelin). Unsaturated double bonds (such as 18:1) were increased in PE and PC.

These changes affect red-cell membrane lipids directly. In red cells, free cholesterol was markedly increased, which decreased the membrane fluidity. However, the concomitant increase in PC leads to an increase in the fluidity, and a decrease in sphingomyelin and in PE also tends to increase the fluidity. 16:0 was increased in total lipids and in PC in the red-cell membrane lipids, which may soften the membranes. Unsaturated double bonds, such as 18:2, were also increased, as a softening effector.

Finally, membrane lipid fluidity was investigated using red-cell membrane liposomes free of cholesterol. The order parameters in ESR showed increased fluidity in the membrane liposomes when free cholesterol was omitted.

In the same way, the total lipid liposomes including free cholesterol were investigated with ESR. The order parameter was completely normal. The ESR spin label studies on the intact red cells also revealed normal membrane fluidity. The results can be interpreted as follows. The increased free cholesterol, which is the primary lesion of this disorder, decreased the membrane lipid fluidity. In contrast to this, phospholipids were changed in nature, counteracting the increased FC. Thus, the overall membrane lipid fluidity was, surprisingly, maintained normally.

The results may suggest the presence of a finer self-adaptive control mechanism counteracting the

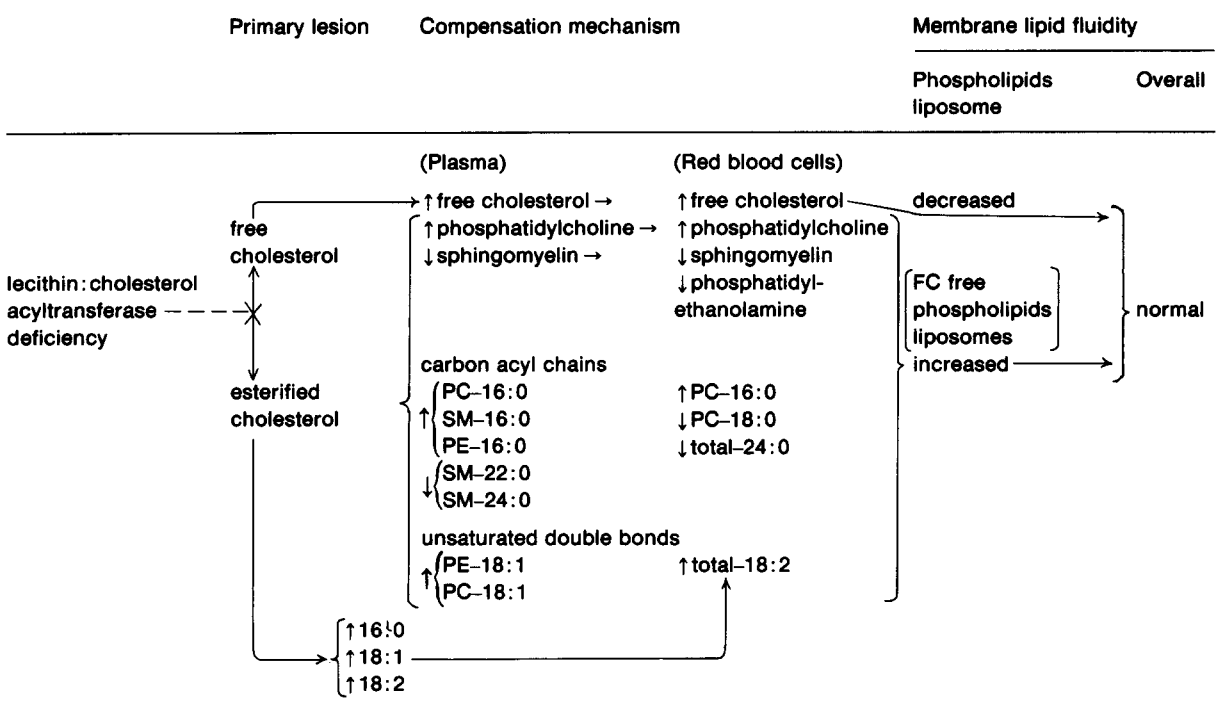

Fig. 2. A proposed mechanism of self-adaptive compensation in red cell membrane lipid fluidity by altering lipid composition in congenital lecithin:cholesterol acyltransferase deficiency. FC, free cholesterol; SM, sphingomyelin.

lipid abnormalities induced by a hereditary metabolic defect.

The ESR spin-label studies were also performed in intact red cells, in addition to the extracted membrane lipids, as mentioned above, in which membrane proteins were stripped from the whole intact red cells. The results in intact red cells showed normal membrane fluidity in the patient. Thus, the increased free cholesterol was also counteracted by self-adaptive softening effectors, which keep the patient's red cell membrane fluidity normal.

Finally, since Ca^{2+} has pronounced effects on membrane fluidity, the possibility exists that divalent cations, especially Ca^{2+}, may be involved in the self-adaptive mechanism, and not merely lipid and fatty acid alterations. Thus, calcium contents in plasma and in red cells were determined in 54 normal subjects and in patients with lecithin:cholesterol acyltransferase deficiency. There were no significant differences in plasma calcium between normal subjects (103 ± 39 μg/ml) and those with lecithin:cholesterol acyltransferase deficiency (98 ± 29), and in red-cell calcium between normal (1.02 ± 0.61 μg/ml red blood cells) and lecithin:cholesterol acyltransferase deficiency (1.13 ± 0.49), respectively. These results indicate that the effects of Ca^{2+} are unimportant, even in the intact red cells.

These results will help in our understanding of the physiological homeoviscous adaptation mechanism in the membrane lipids of patients with altered lipid metabolism.

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