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DECREASED ERYTHROCYTE GHOST MEMBRANE FLUIDITY AS A RESULT OF LIPID COMPOSITION CHANGES IN PLASMA OF ADULT INSULIN-DEPENDENT DIABETICS

Increased microviscosity of erythrocytes membrane ghosts was found in diabetic adult subjects. It seems very likely that membrane cholesterol: phospholipid ratio enhancement is the main reason of decreased membrane fluidity in diabetes. Moreover, plain correlation between membrane cholesterol: phospholipid ratio, plasma cholesterol and membrane fluidity was estimated. The quantitative and structural changes of erythrocyte membranes and plasma lipids quantitative alterations are like to be a reason of diabetic complications arising in poorly controlled diabetes. Significant increase of plasma protein-bound sialic acid content indicates that anormal high concentrations of acute-phase proteins may occur in diabetes. It is likely that mentioned serum sialic acid fluctuations may cause the enhancement of membrane sialic acid content in diabetic red cells.

Introduction

As it is generally accepted presently supramolecular dynamic membrane structure is mostly determined by biochemical membrane composition and the interaction of membrane components [13, 15]. On the other hand, membrane fluidity may regulate structural and reological properties essential for erythrocyte viability. Hence any rigidization of membrane seems to be an important factor reducing red blood cell deformability and the functions of erythrocytes as oxygen carriers [10, 12]. As the cholesterol/ /phospholipid ratio (C/PL) markedly affects membrane microviscosity [4, 14], any quantitative changes of membrane lipids may

disturb physical properties of membrane noticeably [7]. In erythrocytes and platelets with very restricted lipid metabolism, where the phenomena of cholesterol transfer between membranes and plasma serve as a control of cell membrane fluidity, any compositional fluctuations of plasma lipids should be reflected by changed physical parameters of membranes.

The purpose of this study was to find out whether any differences in pyrene lateral mobility in the erythrocyte membranes of adult normal and diabetic patients exist, and to estimate the possible reasons of any discrepances found.

Material and methods

We examined control healthy patients (16-52 years old) and diabetics (21-58 years old) of both sexes. Almost all the diabetics exhibited rather far advanced diabetic complications (as retinopathy and polyneuropathy), all of them were treated with insulin, and disease duration was is range of 0.5-41 years.

Freshly drawn blood collected on 3% sodium citrate was washed three times with 10 mM phosphate buffer saline (pH 7.4). The erythrocyte membranes were obtained by the method of hypotonic hemolysis according to D o d g e et al. [5], and then labelled with pyrene (in methanol solution) to final pyrene concentration of 6.6×10^{-6} M and final lipid phosphorus concentration of 3 µg/ml in each sample.

The fluorescence measurements were performed with Jobin-Yvon spectrofluorimeter at the temperature of 310 K. The fluorescence intensities of pyrene dimer and monomer were measured at 470 nm and 396 nm respectively, and then dimer/monomer ratios were calculated.

Total membrane and plasma cholesterol were determined according to B a b s o n et al. [3], similarly membrane and plasma phospholipids according to R a h e j a et al. [11]. Additionally the levels of total membrane sialic acids as well as total and "free" (it means non-protein bound) plasma sialic acids were estimated according to the method described by W a r r e n [17].

Results and discussion

It turned out that microviscosity of diabetic erythrocyte membranes is significantly enhanced, as well as the content of diabetic erythrocyte membrane cholesterol and C/PL ratio. On the contrary, the amount of erythrocyte membrane phospholipid content diminishes in diabetes, and it is undoubtedly the real reason of the increase in membrane C/PL ratio of diabetic erythrocyte ghosts. Next, the membrane sialic acids is a little, but apparently, elevated. Moreover, plasma cholesterol and phospholipids as well as plasma C/LP ratio are significantly increased (Tab. 1). The level of total plasma sialic acids is highly elevated in diabetic patients and the content of plasma free sialic acids is lowered. The per cent content of fraction of plasma free sialic acids hence might be expected to be diminished in diabetes, and it is really the case.

It seems that quantitative changes of membrane lipids found are one of the main reasons of pyrene motional restriction in hydrophobic bilayer of erythrocyte ghost membranes. This enhancement of erythrocyte membrane microviscosity in diabetes correlates with membrane C/PL ratio ($r = 0.562$) and plasma cholesterol level ($r = 0.553$) and the restrained values of correlation coefficients indicate, that besides of C/PL ratio also other factors as sphingomyelin/lecithin ratio, lipid/protein ratio or unsaturation degree of acyl chains may markedly influence membrane fluidity [14, 15]. The data presented in the table 1 seem to confirm an idea about broadly developed metabolic complications in diabetes. It is not striking that membrane microviscosity enhancement in diabetes correlates positively with the estimated changes in plasma lipids, since diabetic hyperlipoproteinaemia happen to be often accompanied by an accumulation of some plasma lipoprotein fractions [1]. It can also not be excluded that increased membrane sialic acids diminish the membrane fluidity in diabetes.

Strong relation between plasma and membrane C/PL ratios, and particularly the correlation of plasma cholesterol level and membrane fluidity seem to be an unambiguous evidence, that elevated levels of plasma lipids (cholesterol mainly) can seriously af-

Table 1

Statistical comparison of the parameters measured in adult healthy control and in insulin-dependent diabetic subjects

Statystyczne porównanie mierzonych parametrów w kontroli dorosłych i przypadkach cukrzycy insulinozależnej

Parameter	Group	n	$\bar{X}$	SD	Significance (p)
D/M ratio	normal diabetic	25 20	0.679 0.528	0.065 0.038	$p < 0.001$
Membrane cholesterol mM/g protein	normal diabetic	28 28	0.883 1.091	0.144 0.148	$p < 0.001$
Membrane phospholipid mM/g protein	normal diabetic	17 24	0.988 0.780	0.166 0.129	$p < 0.001$
Membrane sialic acid mM/g protein	normal diabetic	17 24	0.119 0.132	0.025 0.015	$p < 0.02$
Plasma cholesterol mM/l	normal diabetic	38 31	6.70 8.93	1.34 1.85	$p < 0.001$
Plasma phospholipid mM/l	normal diabetic	22 23	2.53 3.04	0.35 0.38	$p < 0.001$
Plasma total sialic acids mM/l	normal diabetic	12 23	2.15 3.08	0.13 0.27	$p < 0.001$
Plasma free sialic acids μM/l	normal diabetic	12 23	280.0 217.5	21.3 34.2	$p < 0.001$
% free sialic acid fraction	normal diabetic	12 23	12.96 7.17	1.12 1.36	$p < 0.001$

fect tissue metabolism in diabetes and contribute to the development of diabetic complications.

Though elevated levels of plasma glucose may influence deteriorativelv on the development of such complications [2, 6, 8], we feel sure that the enhanced plasma cholesterol content could cause the complications in the same degree. The type of quantitative changes in plasma lipids found by us corresponds with hyper-

betalipoproteinaemia, which is responsible for arteriosclerosis [1]. Indeed, in examined group of diabetics the microangiopathic changes occur rather as a rule, so then there is much to be said in favour of the hypothesis, that strong positive correlation between plasma levels of some lipids and microangiopathy exists.

The observed quantitative fluctuations of plasma sialic acids in diabetes can be an oblique proof for the occurence of metabolic disturbances in the anabolism of some plasma proteins, mainly so called acute-phase proteins [26]. As reported previously by M c M i l l a n and others the fluctuations of above mentioned proteins may account for plasma viscosity increase and diminished blood flow velocity [9] - the phenomena of particular importance in microcirculation processes.

It would be very desirable, we think, to elucidate the pathomechanism of such disturbances in diabetes, since it is commonly known, that reduced oxygen transport, enhanced hemostasis and erythrocytes or platelets aggregation as well as changed intracellular metabolism of sorbitol and myoinositol together with the effects of enhanced tissue hypoxia in diabetes [8, 9, 13, 18] could by means of interaction with changed blood flow parameters, cause the diabetic microangiopathies.

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OBNIŻONA PŁYNNOŚĆ BŁON ERYTROCYTARNYCH JAKO WYNIK ZMIAN ILOŚCIOWYCH LIPIDÓW OSOCZA W CUKRZYCY INSULINOZALEŻNEJ U LUDZI DOROSŁYCH

W pracy stwierdzono zmniejszoną ruchliwość lateralną pirenu w błonach erytrocytarnych ludzi dorosłych chorych na cukrzycę insulinozależną. Zmianom tym towarzyszyły następujące zmiany ilościowe lipidów błon i osocza: zwiększenie stosunku cholesterol/ /fosfolipidy w błonie w cukrzycy, podwyższenie poziomu cholesterolu w osoczu diabetyków. Jednocześnie wykazano zwiększenie poziomu całkowitych kwasów sjalowych oraz obniżenie związanych z

białkiem kwasów sjalowych w osoczu w cukrzycy. Opierając się na korelacji zmian płynności i zmian ilościowych lipidów, autorzy sugerują, że zmiany powyższe mogą być istotną przyczyną zmian płynności błon w cukrzycy. Obserwowane fluktuacje ilościowe kwasów sjalowych osocza w cukrzycy mogą natomiast świadczyć o występowaniu w cukrzycy zaburzeń metabolicznych w anabolizmie niektórych białek osocza.