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The Problem of Lipid Exchange in Vessel Walls in Insulin Deficit

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Abstract: It has been revealed that in alloxane diabetes the content of the main phospholipids – phosphatidylcholine, phosphatidyletanolamine and phosphatidyl-phosphatidylinosite –decreases in the cells of the vascular wall, whereas the content of the methyl ethers of the fatty acids is not changed, and the content of the fractions of lipids (monoglycerides, diglycerides, triglycerides, cholesterol, free fatty acids, and cholesterol ethers) and phospholipids (phosphatidylserine, cardiolipin, lysophosphatidyletanolamine, lysocardiolipin, sphingomyelin, phosphatide acid and lysophosphatide acid) increase. The depth of these changes correlates with the heaviness of the pathological process.

Key Words: alloxane diabetes, phospholipid, vascular, hypertensive, enzymes

Introduction

Numerous publications concerning functional and morphological changes in the vessel walls taking place during various diseases reveal an idea of the qualitative homogeneity of these changes in the arteries and veins. Therefore, in spontaneously hypertensive rats the shortening of larger and narrower vessels, as well as the narrowing of the capillary-forming vessels in both parts of the channel of a skeletal muscle were recorded (1,2). Impregnation of the vessel walls by blood plasma, its thickening and subsequent fibrosis are recorded both in the arteries (3-5) and in the veins (6). According to vital and posthumous observations of diabetic patients (7), vascular abnormalities (angiopathy) in the retina are expressed in the form of thickening of the basal membrane, microaneurism, edema of walls, and degeneration and atrophy of the endothelium, as well as signs of angiogenesis. Sometimes different levels of manifestation of similar pathologic changes in this or that of the vascular channel are noted; for instance, during the inflammatory process an increase in the lumen and permeability of the microvessels are more expressed in the venous part of the vascular system of an organ (8-9).

Currently, significant attention is paid to structural and functional changes in the cellular membranes during studies of many pathologic states. The level of manifestation and duration of the membrane-damaging processes in an organism determine the clinical heaviness and peculiarities of the progress of many diseases. Membrane destruction as a pathologic phenomenon is predetermined, first and foremost, by the involvement of lipids of cellular membranes in the process of peroxide oxidation of lipids and lipolytic enzymes, which causes a change in the lipid-protein links, and endurance of the cellular metabolism (10-11). However, the importance of the lipid exchange in the development of diabetic microangiopathies is not sufficiently studied, as yet (12).

So far ample data have been amassed on the bioeffector function of lipids and phospholipids both in an organism on the whole, and in a separate cell. Glycosphyngolipids have been revealed to take part in the growth, differentiation and recognition of the cells in intercellular interactions, as well as in the signal transmission. They are antigens and active immune modulators. Simpler sphingolipids and their metabolites (sphingenin, sphingenin-1-phosphate, ceramides) as secondary messengers take part in the process of the growth, differentiation and apoptosis of cells. There are numerous data on the participation of representatives of the phosphoinosite cycle (diacylglycerines, inositolphosphate, inositol-1,4,5-triphosphate and phosphatide acid) as secondary messengers in the process of signalization, which stimulate some forms of protein kinase C and mobilize Ca^{2+} from the intracellular depot, etc. A strong bioeffector is 1-0-alkil-2-acetylphosphatidylcholine (platelet aggregation factor),

which regulates many biological processes in blood. At low concentrations (1-10 mkM), lysophosphatidylcholine stimulates the activity of protein kinase C, aggravates cellular proliferation, stimulates the differentiation of lymphoid cells, etc. Free fatty acids regulate the activity of phospholipases, ion channels, ATPases, G-proteins, protein kinases, model phosphoinositide and sphingomyelin cycles, transfer of the hormonal information, and transcription of genes. Oxylipins are synthesized from polyenic fatty acids as a response to the biological stimulus. Their effects are extremely diverse; they participate in the regulation of most normal and pathologic processes in an organism (13).

The goal of our work was to study regularities in the quantitative changes in the content of various fractions of lipids and phospholipids in the membranes of vascular wall cells in the normal state and under alloxanic diabetes.

Materials and Methods

Observations were made on male rats weighing 180-200 g. Twelve to 16 rats were used in the experiment. Diabetes was caused by a single intraperitoneal injection of alloxan hydrate at a dose of 20 mg per 100 g of body weight (14-15). They were decapitated six days post alloxan hydrate injection. The content of glucose in the blood was determined by the orthotoluidinic method. An acceptable level of glucose is 76 ± 26 mg%.

The lipids from the vascular wall were extracted using the method of Keits (16). Individual lipids and phospholipids were fractionated by horizontal chromatography (17). The percentage of individual lipids and phospholipids was determined by the method of density measuring analysis.

Results and Discussion

The data obtained showed that in alloxanic diabetes the content of phosphatidylcholine, phosphatidyletanolamine and phosphatidylinosite (with the exception of methyl ethers of fatty acids) decreases in the vascular wall whereas the content of all fractions of lipids increases in the vascular wall (Table 1). The number of these lipids significantly increases at the medium and particularly at the heavy stage of diabetes. During the light, medium and heavy stages the level of general phospholipids increases by 7.7, 16.0 and 25.2%, respectively; that of cholesterol increase by 13.7, 26.5 and 39.2%, respectively. The most pronounced increase was that of the concentration of diglycerides, monoglycerides, cholesterol ethers and triglycerides. Hence, at the light stage of diabetes, an increase in the above lipids was 1.83, 1.60, 1.54 and 1.23-fold, respectively; at the medium stage these values were 3.0, 2.1, 2.0 and 1.53-fold; and at the heavy stage they were 4.0, 2.6, 2.73 and 1.71-fold.

Table 1. Content of lipids in the vascular wall of rats with alloxanic diabetes (mkg/mg of tissue; number of experiments 12-16).

Lipids	(Level of glucose in blood, mg %)						
	Healthy rats	Diabetic rats					
		Light		Medium		Heavy	
	76 ± 26	142 ± 28	%	288 ± 32	%	428 ± 49	%
General phospholipids	12.63 ± 0.42	13.61 ± 0.64	107.7	14.66 ± 0.75	116.0	15.81 ± 0.54	125.2
Cholesterol	1.02 ± 0.12	1.16 ± 0.13	113.7	1.29 ± 0.13	126.5	1.42 ± 0.14	139.2
Free fatty acids	1.08 ± 0.05	1.21 ± 0.06	120.0	1.48 ± 0.07	137.0	1.70 ± 0.09	157.4
Methyl ethers of fatty acids	0.37 ± 0.04	0.37 ± 0.05	100.0	0.36 ± 0.04	97.3	0.36 ± 0.04	97.3
Cholesterol ethers	0.41 ± 0.06	0.63 ± 0.07	153.6	0.82 ± 0.06	200.0	1.12 ± 0.08	273.2
Monoglycerides	0.10 ± 0.02	0.16 ± 0.03	160.0	0.21 ± 0.05	210.0	0.26 ± 0.06	260.0
Diglycerides	0.06 ± 0.01	0.11 ± 0.02	183.3	0.18 ± 0.04	300.0	0.24 ± 0.05	400.0
Triglycerides	1.03 ± 0.07	1.27 ± 0.12	123.3	1.58 ± 0.16	153.4	1.76 ± 0.12	170.9
Cholesterol/phospholipid	0.0807	0.0852	105.5	0.0879	108.9	0.0898	111.2

The 1,2-diglycerides are known to produce a significant effect on the bilayer lipids. As model experiments have shown (18-19), they cause damage to bilayer organization and the appearance of an isotropic phase in membranes, which was confirmed by nuclear magnetic resonance (19). A decrease in the content of monoglycerides and diglycerides causes the appearance of heavily curved sites in the bilayer, which form folds and intramembranic particles (18). It has long been established that any defects in the bilayer organization of a membrane are preferable sites the hydrolytic action of phospholipases and proteases irrespective of the reasons causing them (21).

Accumulation of general phospholipids in the vascular wall of the diabetic animals took place owing to the minor components of the phospholipid composition (Table 2), but not to the major fractions of phospholipids. While analyzing the quantitative content of the individual phospholipids in the vascular wall in the diabetic animals there was a sufficient decrease in the levels of phosphatidylcholine, phosphatidyletanolamine and phosphatidylinosite. The level of changes in the content of phospholipids depended on the diabetes heaviness. Hence, if at the light stage of diabetes the level of the above mentioned phospholipids decreased only by 3.2, 2.8 and 5.1%, at the medium stage it decreased by 10.2, 11.6 and 12.8%, and at the heavy stage by 14.7, 16.1 and 18.0%, respectively. A decrease in phosphatidylcholine and phosphatidyletanolamine was accompanied by a significant increase in the content of their monoacylic forms and free fatty acids. Against the norm at the light, medium and heavy stages of diabetes, the level of lysophosphatidylcholines increases 1.17, 1.63 and 2.08-fold; lysophosphatidyletanolamines 1.16, 1.33 and 1.53-fold; and free fatty acids 1.20, 1.37 and 1.57-fold.

Conclusion

An excess accumulation of lysoforms of phospholipids and free fatty acids in the cells of the vascular wall in diabetes causes deep abnormalities in the structure and the function of biological membranes (21). Samartsev has shown that high concentrations of fatty acids block the transport of ATP and ADP through the inner membrane of mitochondria, inhibiting adenine nucleotide translocase.

Table 2. Content of phospholipids in the vascular wall of rats with alloxanic diabetes (mkg/mg, number of experiments 12-16).

Lipids	(Level of glucose in blood, mg %)						
	Healthy rats	Diabetic rats					
		Light		Medium		Heavy	
	76 ± 26	142 ± 28	%	288 ± 32	%	428 ± 49	%
Phosphatidylcholine	5.12 ± 0.11	4.96 ± 0.19	96.8	4.60 ± 0.14	89.8	4.37 ± 0.13	85.3
Phosphatidyletanolamine	2.24 ± 0.08	2.20 ± 0.09	98.2	1.98 ± 0.07	88.4	1.88 ± 0.06	83.9
Sphingomyelin	1.39 ± 0.06	1.59 ± 0.06	114.4	1.76 ± 0.07	126.6	2.18 ± 0.12	156.8
Phosphatidylserine	0.42 ± 0.05	0.56 ± 0.06	133.3	0.69 ± 0.04	164.3	0.84 ± 0.05	200.0
Phosphatidylnosite	0.39 ± 0.04	0.37 ± 0.03	94.9	0.34 ± 0.03	87.2	0.32 ± 0.02	82.0
Cardiolipin	1.12 ± 0.08	0.37 ± 0.03	130.3	2.00 ± 0.11	178.6	2.46 ± 0.10	219.6
Lysophosphati-dyletanolamine	0.42 ± 0.04	0.49 ± 0.04	116.6	0.56 ± 0.05	133.3	0.64 ± 0.06	152.4
Lysocardiolipin	0.11 ± 0.02	0.13 ± 0.02	118.2	0.16 ± 0.02	145.4	0.18 ± 0.01	163.6
Phosphatide acid	0.25 ± 0.03	0.39 ± 0.04	156.0	0.48 ± 0.05	192.0	0.66 ± 0.04	264.0
Lysophosphatide acid	0.21 ± 0.04	0.42 ± 0.05	200	0.64 ± 0.07	304.7	0.82 ± 0.06	390.5
Phosphatidylcholine/ phos-phatidyletanolamine	2.28	2.25	98.7	2.42	106.1	2.32	101.7
Phosphatidylcholine/ lysophosphatidylecholine	11.13	9.18	82.5	6.64	59.6	4.55	40.9
Phos-phatidyletanolamine	5.33	4.49	84.2	3.53	66.2	2.94	55.1

A decrease in phosphatidylcholine, phosphatidyl etanolamine and phosphatidylinosite and an increase in their monoacylic form of free fatty acids in the vascular wall in diabetes is likely to be connected with the violation of processes of the transacylation of fatty acids into the above mentioned phospholipids due to inhibition of the acylase response against the activation of phospholipase A. This results in inhibition of the synthesis of the acylic groups of the above phospholipids.

An increase in the content of phosphatidyl serine in the cells of the vascular wall of diabetic animals (at the light, medium and heavy stages, 1, 1.33 and 1.64, respectively) against a decrease in the content of phosphatidylcholine, phosphatidyletanolamine is perhaps a result of the enzymatic responses of methylation and decarboxylation taking place in the cell membranes (22). It can be assumed when there is an insulin deficit the process of transformation of phosphatidylserine into phosphatidyletanolamine (reaction of decarboxylation) decelerates, which in turn causes the deceleration of the transformation of phosphatidylserine into phosphatidylcholine (reaction of methylation).

A characteristic feature of changes in the vascular walls in experimental diabetes is a sharp increase in the content of cardiolipin. At the light, medium and heavy stages of diabetes, the content of cardiolipin increases 1.30, 1.79 and 3.20-fold, respectively, in comparison with the norm. It is known that mitochondria have a full set of enzymes for the synthesis of cardiolipin, which are connected with the inner membrane (21,23). In our opinion, an increased content of cardiolipin in the vascular wall of diabetic animals is connected with the strengthening of the activity of diglyceride-glycero-5p-phosphatidyltransferase, phosphatidyl glycerophosphatase and cardiolipin synthetase. An increase in the concentration of lysocardiolipins in the vascular wall of diabetic rats (at the light, medium and heavy stages of the pathology 1.18, 1.45 and 1.64-fold, respectively, of the norm) against an increase in the amount of cardiolipin, in our opinion, is a result of an increase in the catalytic activity of phospholipase A.

When there is a deficit of insulin in the animal organism, the content of sphingomyelin, phosphatide acid and lysophosphatide acid increases in the cells of the vascular cell depending on the heaviness of the pathological process. Hence, the level of sphingomyelin at the light, medium and heavy stage of diabetes increases 1.14, 1.27 and 1.57-fold; phosphatide acid 1.56, 1.92 and 2.64-fold; and lysophosphatide acid 2.0, 3.0 and 3.9-fold of the initial value. In diabetes, an increase in phosphatide acid, especially lysophosphatide acid, in our opinion is connected with the increase in the catalytic activity of phospholipase D. This enzyme is known to carry out the hydrolysis of phospholipids and lysophospholipids of cytoplasmic membranes and the membranes of cell organelle with the formation of phosphatide acid and lysophosphatide acid, as well as free bases (23).

A decrease in the neutral phospholipids results not only in the accumulation of lyso derivatives, but also to the redistribution of the surface charge of the membrane owing to a sharp growth of phosphatidylcholine, cardiolipin and phosphatide acid, which have a negative charge. This restructuring of the lipid content in the membranes of the vascular wall can significantly strengthen the adhesion of the form elements of blood in diabetic patients. The thickening of the vascular wall in diabetic patients is likely to be connected with the deposit of lipid fractions in it. The other adverse factor is an accumulation of cholesterol, which can significantly increase the level of microviscosity of the tissue cell membranes in the vascular wall at diabetes.

In diabetes, the ratio of cholesterol/general phospholipids, which plays an important role in the preservation of the membrane integrity, increases and changes depending on the heaviness of the pathological process. The ratio of phosphatidylcholine/phosphatidyletanolamine under similar conditions does not practically differ from the norm. At the same time, the ratio of diacylic and monoacylic forms of phospholipids, which play an important role in the preservation of the integrity of cell membranes and their organoids in diabetes, decreases and correlates with the heaviness of the pathological process. Therefore, if at the light stage of diabetes the ratio of phosphatidylcholine/lysophosphatidylcholines and phosphatidyletanolamine/lysophosphatidyletanolamine decreases only 1.17 and 1.45-fold of the norm, this implies that with the aggravation of diabetes the process of damage in cell membranes in the vascular wall significantly increases.

Thus, it is believed that changes in the cells of vascular wall tissues during insulin deficit in an organism are due to the emergence of lesions manifested in the violation of

lipid and phospholipid exchange, which is expressed in a decrease in the phosshatidylcholine, phosphatidyletanolamine and phosphatidylinosite content and in the increase in the other fractions of lipids and phospholipids with the preservation of the concentration of methyl fats of fatty acids. The intensity of these changes is proportional to the heaviness of the diabetic state.

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References

1. Engelson ET, Schmidt-Schonbein GW, Zweifach BW. The microvasculature in skeletal muscle. III. Venous network anatomy in normotensive and spontaneously hypertensive rats. J. Microcirc. Clin. Exp 4:, 229-248, 1985.
2. Engelson, Schmidt-Schonbein GW , Zweifach BW. The microvasculature in skeletal muscle. Arteriolar network anatomy in normotensive and spontaneously hypertensive rats. Micro VSC Res 1: 31 356-374, 1986.
3. Simionescu M, Simionescu N, Palade GE. Segmental differentiations of cell functions in the vascular endothelium. Arteries and Veins. J. Cell Biol. 68: 705-723, 1976.
4. Winguist RJ, Webb RC, Bohr DF. Vascular smooth muscle in hypertension. Fed. Proc 41: 2387-2393, 1982.
5. Ross R. The pathogenesis of athesclerosis – an update. New Engl. L. Med 314: 488-500, 1986.
6. Greenberg S, Wilborn W. Functional and structural changes in veins in spontaneous hypertension. II. Arch. Int. Pharmacodium 258: 2 208-233, 1982.
7. Monschot WA., Lee WR. New vessel formation in diabetic and non-diabetic retinal vasculopathy. II. Diabetes and metabolism 14: 535-539, 1988.
8. Chernyh AM. Osobennosti venoznogo zvena mikrotsirkulatornoi pochechnoi i gormonalnoi gipertonii. Summary of dissertation, Moscow 25 1976. (In Russian).
9. Shoshenko KA. Reaktsii arterialnyhi venoznyh mikrososudov pri gipertenzii i alloksanovom diabete u krys. Physio. J. USSR after Sechenov 78: 4, 46-53. (In Russian).
10. Aleksandrovsky Ya A. Molekulyarnye mehanizmy razvitiya diabeticheskih oslozhnenij. biochemistry, V.63, Issue 11, p.1470-1479, 1998. (In Russian).
11. Almatov KT. Mehanizmy razvitiya povrezhdenij membran mitohondrij i rol lipoliticheskoi sistemy. Dissertation for Doctor of Sciences degree, Tashkent 410 1990. (In Russian).
12. Akbarov ZS, Turakulov Ya H, Kasimova GM, Mirtalipov DT, Abidov AA. Lipidnyj sostav sosudistoj stenki bolnyh saharnym diabetom s mikroangiopatiyami. Doklady Akademii Nauk, 3: 59-60, 1991. (In Russian).
13. Dyatlovitskaya EV, Bezuglov VV. Lipidy kak bioeeffektory. Introduction. Biochemistry 63: Issue 1, 3-5, 1998. (In Russian).
14. Almatov KT, Agzamov H, Rahimov MM. Turakulov Ya H. Izucheniye funktsionirovaniya mitokhondrii pecheni pri alloksanovom diabete. Voprosy med. Himii, E. 29, Issue 1, 1-65, 1983. (In Russian).
15. Lenzen S, Panten U. Alloxan: history and mechanism of action. Diabetologia 31: 6, 337-342, 1988.
16. Keits M. Tehnika lipidologii, vydeleniye, analiz i identifikatsia lipidov, Moscow, Mir Publishers, 321, 1975. (In Russian).
17. Kargapolov AV, Analiz lipidnogo sostava mitohondrialnyh I endoplazmaticheskih membran s pomoshch'yu metoda protochnoi gorizontalnoi hromatografii. Bioorganic Chemistry 46: 4, 691-698, 1981. (In Russian).
18. Sorokumova GI, Vasilenko IA, Shvets VI, Selintseva AA, Borovyagin VL. Vliyanie metabolitov fosfolipidov na structuru modelnyh membran. Bioorganic Chemistry, 9: 8, 1106-1111, 1983. (In Russian).
19. Shragin AS, Vasilenko IA, Selintseva AA, Shvets VI. Vliyanie metabolitov fosfolipidov na sliyanie modelnyh membrasn razlichnogo sostava. Boil. Membrany, ZV. 2: 8, 789-794. (In Russian).
20. Dawson RM, Irvine RF, Bray J, Quinn PJ. Long-chain unsaturated diacylglycerols cause a perturbation in the structure of phospholipid bilayers rendering them susceptible to phospholipase attack. Biochem. Biophys. Res. Com 125: 2, 789-794, 1984.
21. Almatov KT. Fermentativnye prevrashcheniya fosfolipifov membran mitohondrij. Tashkent: Universitet, 30 pp., 1993. (In Russian).
22. Bereziat C. Renousvellcment des acides gras des membranes cellulaires. Ann. Nutr. Et alim 34: 2, 241-254, 1989.
23. Lyakhovich VV. Problemy sinteza fosfolipidov v mitohondriah. Biological Sciences 2: 36-45, 1976 (In Russian).