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CHANGES IN SOME PARAMETERS OF LIPID METABOLISM IN ERYTHROCYTE MEMBRANES DURING DEVELOPMENT OF ALLOXAN DIABETES

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Cell membranes are kept in the intact state by many factors, among which the most important are normal functioning of certain membrane-bound lipid-dependent enzymes, on the one hand, and a continuous process of lipid renewal through maintenance of a strictly limited level of lipid peroxidation (LPO), on the other hand [2-4, 7, 11, 13].

The aim of this investigation was to study the molecular mechanisms of the action of LPO products on the functional state of a biological membrane during alloxan diabetes (AD). The general principles governing development of changes in the qualitative and quantitative composition of phospholipids (PL), concentrations of total, free, and esterified cholesterol (TCh, FCh, and ECh, respectively) and of α-tocopherol (TP), and the cholesterol/phospholipid (Ch/PL) ratio in the erythrocyte membrane (EM) were studied.

EXPERIMENTAL METHOD

Experiments were carried out on 100 noninbred male albino rats weighing 180-200 g, kept on an ordinary diet. AD was induced by the usual method: intraperitoneal injection of alloxan in a dose of 15 g/kg body weight. Blood taken for investigation was stabilized with oxalate. The blood glucose concentration was determined by the orthotoluidine method. EM were isolated, purified, and identified by Limber's method [14], and PL were determined by one-way ascending chromatography on Filtrak FN-11 paper (East Germany), impregnated with silicic acid [6].

The intensity of LPO was judged by the accumulation of malonic dialdehyde (MDA) in enzymic and nonenzymic systems of LPO [3]. Concentrations of TCh, ECh, and FCh were determined by a unified method [10], TP and EM by Duggan's method [12], and protein by Lowry's method [15].

EXPERIMENTAL RESULTS

All periods of development of AD were characterized by appreciable activation of LPO in EM (Table 1). Release of LPO products definitely predominated on the 7th day of the disease in the NADPH-dependent system (by about 66%), and there was a smaller shift (by about 43%) in the ascorbate-dependent system of LPO. On the 14th day of the disease, release of MDA in the above peroxidation systems continued to rise, up to about 145 and 106%, respectively. On the 21st day after injection of alloxan a tendency was noted for the intensity of LPO to decrease, although as before the MDA level in the two systems was higher than that in the control animals. It can thus be concluded from the results that lipid peroxidation takes place with high activity in EM of albino rats with AD, determined at all times of observation.

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TABLE 1. LPO Activity in Erythrocyte Membranes (in mmoles MDA/mg protein) and TP Concentration (in μg/mg protein) during AD (M ± m)

Parameter	Control	7th Day	14th Day	21st Day
ADP	2,21±0,08	3,17±0,12*	4,56±0,23*	3,74±0,16*
NDP	2,23±0,01	3,7±0,09*	5,46±0,29*	4,32±0,24*
TP	2,87±0,05	1,02±0,26*	1,87±0,09*	2,21±0,19*

Legend. *p < 0.05 compared with control. ADP and NDP) Ascorbate-dependent and NADPH-dependent LPO, respectively.

TABLE 2. Changes in Phospholipid Content (in μg lipid phosphate/g dry residue of EM) and Cholesterol (in μg/mg protein) in EM during AD (M ± m)

Parameter	Control	7th Day	14th Day	21st Day
Phospholipids:				
Neutral	680±14	1800±6	1230±16	1300±15
Acid	10±10	590±93	230±11	410±11
Total	990±2	2390±64	1360±14	1710±12
Cholesterol				
Total	55,7±3,0	67,2±1,4	80,8±2,3	64,6±1,2
Free	30,6±0,8	50,3±1,6	62,3±2,1	43,2±1,9
Esterified	25,2±0,3	17,0±0,6	18,5±2,1	21,4±2,2
TCh/PL	0,056	0,037	0,066	0,042

The changes observed in the TP level in EM may play an important role in the explanation of the mechanisms of development of the disturbances described above. The TP level, sharply lowered on the 7th day of development of the disease (by about 74%), recovered a little by the 14th and 21st days, but still remained 40 and 23%, respectively below the control level (Table 1). These results can be explained by the unique manifestation of the compensatory reaction of the body to the abnormal conditions of its existence, as a result both of partial restoration of function of the alloxan-damaged β-cells, namely intensification of granulation and secretion formation in them, and the de novo formation of small islets of Langerhans, consisting perhaps of only β-cells [1].

The increased background intensity of peroxide formation during AD adversely affects the functional activity of the biological membrane, and in particular, the reaction of membrane permeability. An essential role in the stabilization of structural and functional integrity of the biomembrane is played by PL, which are known to be a substrate for LPO. Marked quantitative and qualitative changes discovered in the PL level in EM, and the fluctuating time course of the levels of these compounds observed at different stages of development of the pathological process, reflect the complexity of the biochemical processes catalyzing their synthesis and breakdown. The increase in the PL concentration discovered on the 21st day of development of AD, by about 81% compared with the control, was linked with intensification of degradation of lipid-protein complexes. The possibility cannot be ruled out that under these circumstances PL exchange between blood lipoproteins and EM also is intensified. The shift observed was due to an increase in the concentration of both neutral and acid PL; the changes in the level of the former were more marked. In all probability, such substantial deviations in quantitative levels of PL, especially of PL-glycerides, are based on an essential metabolic trend. Under energy deficiency conditions, which characterize diabetes, PL may to some extent play the role of additional oxidation substrates, thereby performing the function of compounds involved in the compensatory and adaptive reactions of the body.

AD is characterized by marked changes in TCh, FCh and ECh levels (Table 2). The raised levels of TCh found in EM (by about 21%) on the 7th day continued to rise until the 14th day (45%), but they fell appreciably until the 21st day, although still remaining about 16% higher

than the control level. A similar rule was discovered also in the changes in FCh concentration; so far as ECh is concerned, its level fell sharply on the 7th day of the disease, and thereafter remained below the control value. The shifts described above are probably due to changes in cholesterol exchange between very low-density lipoproteins and high-density lipoproteins (HDL) in EM, which plays an important role in the regulation of the phasic state of the cell membrane lipids [5, 9], and also to intensification of LPO in low-density lipoproteins, induced by HDL [8]. The abrupt character of the changes in total of PL and TCh is reflected in the value of the TCh/PL ratio, and it can serve to some degree as an indicator of destruction of EM taking place and of disturbances of cell membrane function developing as a result.

Analysis of the experimental data leads to the conclusion that AD is characterized by profound disturbances of the structural architectonics of the cell membrane and of its functional activity. Among the many pathogenetic factors involved in the molecular mechanism of these disturbances, an important place is occupied by overactivation of LPO processes, with the release of large quantities of toxic products, which have a powerful memranolytic action. The interdependence and diametrically opposite direction of shifts in the antioxidative activity of the lipids in the systems of the animals studied, due to the presence of the natural antioxidant TP, and the intensity of the course of LPO processes must be emphasized.

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