Modification of Transport Function of Plasma Albumin during Atherosclerosis and Diabetes Mellitus

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Parameters characterizing the processes of association, transport, and dissociation of fatty acid molecules on the corresponding binding sites of plasma albumin in patients with atherosclerosis and diabetes mellitus were studied by electron paramagnetic resonance method. In these patients transport function of albumin differed from normal. It should be emphasized that these differences were specific for atherosclerosis and diabetes mellitus, which is of considerable diagnostic value.

Key Words: *albumin; fatty acids; atherosclerosis; diabetes mellitus; electron paramagnetic resonance*

Disturbances in lipid metabolism, in particular, metabolism of unsaturated fatty acid, play an important role in the pathogenesis of various diseases, including atherosclerosis and diabetes mellitus [1,2]. Albumin, the major carrier of fatty acids in the blood, has specific binding sites for these molecules. Albumin transports not only fatty acids, but also several endogenous metabolites (bilirubin and hematin), drugs, and poisons [5]. Since binding sites are formed by the tertiary structure of albumin, conformational changes in the protein globule after binding of various substances violate its ability to transport other ligands. These shifts can provide the basis for the development of various pathologies. In some diseases overproduction and high-affinity binding of some substances to albumin impair transport of other metabolites. Moreover, some drugs can act as blockers [5]. Conformational changes in albumin molecule can be detected by various physicochemical methods, *e.g.*, electron paramagnetic resonance (EPR) spectrometry.

MMS analysis (EPR spectrometry with spin probe based on 16-doxyl-stearic acid) allows evaluation of several parameters characterizing transport function and conformational state of albumin molecules. This method is highly informative in the diagnostics of various tumors [3,4]. In the present work blood plasma from patients with atherosclerosis and diabetes mellitus (25-50 years) was studied to determine the conformational state and transport function of albumin.

MATERIALS AND METHODS

Commercial 16-doxyl-stearic acid (Sigma-Aldrich GmbH) served as a marker molecule (spin probe). This long-chain stearic fatty acid is labeled with nitroxyl spin label in the 16-position. Ethanol was used as a polar reagent inducing conformational changes in fatty acid-binding sites.

Blood plasma from patients with atherosclerosis and diabetes mellitus served as a biological material. Venous blood was fractionated at 150 rpm for 25 min.

Eight microaliquots were prepared from each plasma sample by mixing specified amounts of spin-labeled stearic acid, ethanol, plasma, and distilled water in wells of a special microplate (Table 1). The samples were incubated at 37°C for 10 min under constant mixing. After incubation these mixtures were transferred into glass capillaries (KABE GmbH) and EPR spectra were recorded.

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TABLE 1. Volumes and Final Concentration of Components in the Mixture for EPR Analysis

Component	Mixed volumes, μl							
Plasma	50	50	50	50	50	50	50	35
Water	—	—	—	—	—	50	50	15
Ethanol	10	12	14	14	14	12	14	10
	Final concentration							
Ethanol, M	2.90	3.37	3.80	3.80	3.80	1.86	2.14	2.90
Probe, 10^{-3} M	0.83	1.61	2.34	1.09	1.82	0.89	1.31	1.78

We used an EPR analyzer and Mobility of Molecular Structure software (MMS). Binding of the spin probe, polarity of the microenvironment, and mobility of various fatty acid binding sites were determined. The spectra were automatically analyzed using original software [3].

Transport function of albumin was determined by its interaction with spin-labeled molecules, polarity of the microenvironment, and conformational mobility of albumin-binding sites. We evaluated the binding constant (KB); capacity of the first binding site (N1 0); ratio between capacities of the first and second binding sites (R2); limit of conformation stability for albumin (L2) equal to the number of fatty acid molecules in major sites that provide opening of the BS-2 site during interaction with target cells in metabolic transport of albumin; albumin capacity in various phases of transport (NT 0, NL 0, and NU 0: transport in the vascular system and LCFA load (adsorption) and removal (dissociation), respectively); integral constants for albumin binding during transport and adsorption (KT and KL); constants of dissociation characterizing its efficiency in the corresponding phase (DU); and ratio between potential/integral transport effectiveness (ITE) and real transport effectiveness of albumin (RTE).

RESULTS

Transport function of albumin was changed in patients with atherosclerosis and diabetes mellitus. KB, KT, and KL markedly increased in patients with diabetes mellitus, which attested to the presence of other bound ligands modifying albumin conformation and increasing availability of active binding sites for fatty acids (Table 2). By contrast, in atherosclerosis binding constants decreased, which attested to reduced availability of binding sites for fatty acids. Modification of the tertiary structure in biopolymers accompanying the formation of ligand-protein complexes induces conformational changes in regions of the protein molecule interacting with other ligands. The binding capacity of these centers can decrease or increase (negative and positive cooperativity of binding sites) [3]. The dissociation constant increased in atherosclerosis, but decreased in diabetes mellitus.

Transport capacity of albumin (NTO) markedly decreased in diabetes mellitus, but did not differ from the control in atherosclerosis.

The study of flexibility revealed considerable changes in conformational mobility of albumin molecules in the plasma from patients and healthy donors (Table 2). Mobility of the second binding site (K2)

TABLE 2. Binding Capacity, Conformation, and Effectiveness of Fatty Acid Transport by Albumin

Parameter		Normal	Atherosclerosis	Diabetes mellitus
Binding capacity	KB	8.4	5.5±2.1	22.0±9.7
	KT	150.4	104.1±32.8	223.0±86.7
	KL	150.4	104.1±32.8	223.0±86.7
	NT0	19.3	20.3±0.7	10.7±2.6
	DU	0.057	0.095±0.030	0.040±0.010
Conformational mobility	R2	0.870	0.937±0.050	0.799±0.320
	K2	1.490	0.579±0.640	1.489±0.770
	L2	2.430	7.303±3.450	1.954±1.650
Transport effectiveness, %	ITE	100.0	120±14	64±26
	RTE	100.0	56±30	36±14

3-fold decreased in atherosclerosis, which attests to the presence of a large high-affinity ligand preventing conformational changes that albumin molecule. Moreover, we revealed a considerable increase in the limit of conformation stability for albumin (L2), which is equal to the number of fatty acid molecules in the major sites that provide opening of the BS-2 site during interaction with target cells. By contrast, this parameter decreased in diabetes mellitus.

Real transport effectiveness of albumin (RTE) in atherosclerosis and diabetes mellitus decreased by 30-40 and 60-70%, respectively (Table 2).

Our study demonstrated modification of albumin molecule and changes in its transport characteristics in atherosclerosis and diabetes mellitus. The observed changes are specific for atherosclerosis and diabetes mellitus. This method can provide the basis for the development of new test systems for the diagnostics and therapeutic monitoring of atherosclerosis and diabetes mellitus.

REFERENCES

1. A. Sh. Zaichik and L. P. Churilov, *Basic Principles of Pathological Chemistry* [in Russian], St. Petersburg (2000).
2. A. N. Klimov and N. G. Nikul'cheva, *Lipids, Lipoproteins, and Atherosclerosis* [in Russian], St. Petersburg (1995).
3. G. Matthes, G. Seibt, and V. Muravsky, *Analysis of Plasma Proteins and Tumor Screening Diagnostics Using ESP. New Drugs*, No. 2, 26-32 (2001).
4. G. Matthes, G. Seibt, V. Muravsky, *et al.*, *Transfus. Apheresis Sci.*, **27**, No. 2, 129-135 (2002).
5. T. Jr. Peters, *All about Albumin*, New York (1996).