ONCOLOGY

Lipid Peroxidation in Erythrocyte Membranes of Women with Benign and Malignant Neoplasms of the Endometrium

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The content of primary and secondary products of membrane peroxidation, total cholesterol concentration, and relative microviscosity of erythrocyte membranes were measured in women with endometrial cancer and uterine leiomyoma. The content of conjugated dienes in erythrocytes was highest in female patients with malignant tumors of the endometrium. The relative microviscosity of erythrocyte membranes in patients with uterine leiomyoma and endometrial cancer was elevated in the zone of lipid—lipid and protein—lipid contacts.

Key Words: *erythrocyte; lipid peroxidation; relative microviscosity of membranes; uterine leiomyoma; endometrial cancer*

A large body of evidence indicates that LPO play a role in carcinogenesis. Studying the mechanisms associated with free radical oxidation (FRO) of lipids during tumor development is an urgent problem [1,8,11,12]. FRO has a variety of effects on biological membranes. They can be divided into the following two groups: the effects associated with oxidation of unsaturated fatty acids in membrane phospholipids (group 1) and interaction of reactive oxygen species and LPO products with membrane proteins (group 2). These effects result in the appearance of "peroxide clusters" in the lipid bilayer. It is followed by an increase in the relative microviscosity of membranes, decrease in molecular mobility of phospholipids, changes in the barrier properties and membrane permeability, and impairment of hormone-receptor interactions and intracellular signal transduction. Under pathological conditions, these changes contribute to microcirculatory disorders and hypoxia of organs and tissues [4].

Here we measured the content of primary and secondary products of LPO, total cholesterol concentration, and relative microviscosity of erythrocyte membranes in women with endometrial cancer and uterine leiomyoma.

MATERIALS AND METHODS

One hundred and five women (32-79 years) were examined in the Regional Oncology Center. There were patients with uterine leiomyoma (19 women, 32-65 years) and endometrial cancer (86 women, 32-79 years). The control group included 32 women of similar age. Erythrocytes were isolated from the venous blood. Heparin was used as an anticoagulant. Hemoglobin concentration in packed erythrocytes was measured by the standard cyanhemoglobin method.

The content of conjugated dienes (CD) was measured in a heptane extract at 233 nm [6]. MDA concentration was estimated in the reaction with TBA in

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an acid medium [4]. The relative microviscosity of erythrocyte membranes was evaluated by the method of lateral diffusion of a hydrophobic probe pyrene ($C_{16}H_{10}$). Microviscosity of the membrane lipid bilayer and zone of protein-lipid contacts was recorded at 334 and 286 nm, respectively. Fluorescence of pyrene dimers and monomers was studied on an Aminco Bowman Siries 2 spectrofluorometer (Thermo Spectromic) [2]. Total cholesterol concentration was measured by the method of Liberman–Burkhard with modifications of Il'k [3].

The data are expressed as the arithmetic mean (*M*) and error of the mean (*m*). The hypothesis of statistical significance was verified by the Mann–Whitney test. Statistical processing was performed with Statistica 7.0 software (StatSoft Inc.).

RESULTS

The content of CD increased by 20%, while the concentration of MDA decreased by 30% in erythrocytes from patients with uterine leiomyoma (as compared to the control; Table 1). Similar changes were observed in erythrocytes from patients with endometrial cancer. The content of CD increased by 38%, while the concentration of MDA decreased by 49% in erythrocytes from patients with endometrial cancer.

MDA is a bifunctional cross-linking reagent. This compound is capable of binding to NH_2 groups of amino acids, proteins, and nitrogen bases. Peroxidation of erythrocyte membranes is elevated in patients with uterine leiomyoma and endometrial cancer. This conclusion was derived from an increase in the content of CD (primary products of LPO). A decrease in MDA concentration under these conditions could be related to the following two reasons: (1) increase in MDA binding to the membrane (measurement of free MDA); and (2) activation of aldehyde dehydrogenase that plays a role in metabolic transformations of MDA in erythrocytes [7].

The accumulation of primary products of LPO attenuates the hydrophobic bonds in cell membranes and increases the relative microviscosity of erythrocyte membranes. We showed that the relative microviscosity of erythrocyte membranes from patients with uterine leiomyoma increases in the zone of protein—lipid interactions and lipid bilayer (by 218 and 185%, respectively). The index for viscoelastic properties of erythrocyte membranes from patients with endometrial cancer was shown to increase in the zone of protein—lipid and lipid—lipid interactions (by 73 and 51%, respectively, compared to erythrocytes from healthy women; Table 2).

The relative microviscosity of membranes is an integral criterion, which depends on some factors (unsaturation of lipids, phospholipid composition, membrane protein content, degree of LPO, and membrane cholesterol concentration) [5,9,10]. Cholesterol con-

TABLE 1. Amount of LPO Products in Erythrocytes from Women with Malignant and Benign Neoplasms (*M*±*m*)

Parameter	Clinical group		
	control (*n*=32)	uterine leiomyoma (*n*=19)	endometrial cancer (*n*=86)
CD, rel. units	2.16±0.14	2.59±0.58*	2.99±0.10**
MDA, μmol/ml erythrocytes	8.11±0.19	4.98±0.05**	4.15±0.13**

Note. *p<0.05 and **p<0.001 compared to the control.

TABLE 2. Relative Microviscosity of Erythrocyte Membranes from Women with Malignant and Benign Neoplasms (*M*±*m*)

Parameter	Clinical group		
	control (*n*=32)	uterine leiomyoma (*n*=19)	endometrial cancer (*n*=86)
Microviscosity (lipid bilayer), F_M/F_E	0.73±0.40	2.08±0.04*	1.10±0.05+
Microviscosity (protein—lipid interaction), F_M/F_E	0.45±0.06	1.43±0.06*	0.78±0.06+

Note. F_E, fluorescence of excimers (λ_{em}=470 nm); F_M, fluorescence of monomers (λ_{em}=395 nm). *p<0.001 compared to the control; +p<0.01 compared to uterine leiomyoma.

TABLE 3. Total Cholesterol Concentration in Erythrocyte Membranes from Women with Malignant and Benign Neoplasms (*M*±*m*)

Parameter	Clinical group		
	control (*n*=32)	uterine leiomyoma (*n*=19)	endometrial cancer (*n*=86)
Total cholesterol, $\times 10^{-9}$ g/1000 erythrocytes	4.1±0.3	5.2±0.2*	4.7±0.1*

Note. *$p<0.001$ compared to the control.

centration was measured to evaluate the cause of an increase in the relative microviscosity of membranes.

Cholesterol concentration in female patients with uterine leiomyoma and endometrial cancer was elevated by 27 and 15%, respectively, compared to the control (Table 3). Excess cholesterol increases the relative microviscosity of membranes, which reduces the rate of reactions with diffusion-dependent limiting stages. Membrane cholesterol has a negative effect on lipid mobility and increases the mechanical strength of a bilayer [9].

Our results illustrate the opposite changes in the content of primary and secondary products of LPO in erythrocyte from patients with uterine leiomyoma and endometrial cancer (increase in the content of CD and decrease in the concentration of MDA). The relative microviscosity of erythrocyte membranes in female patients with uterine leiomyoma and endometrial cancer was elevated in the zone of lipid—lipid interactions and, particularly, in the zone of protein—lipid interactions. These changes are probably related not only to the increased concentration of cholesterol in erythrocytes, but also to the modification of membrane proteins by MDA. We conclude that this disorder is accompanied by significant structural and functional changes in erythrocyte membranes of patients with endometrial cancer. They are associated with the activation of LPO and increase in total cholesterol concentration, which results in a change in viscoelastic properties of erythrocyte membranes.

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