In Vitro Effect of Fucose-Specific Lectins on Rat Erythrocyte Membranes under Normal Conditions and during Toxic Stress

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We studied the effect of various fucose-specific lectins on lipid peroxidation in rat erythrocyte membranes under normal conditions and during exposure to the toxic agent. Under normal conditions parameters of lipid peroxidation in erythrocyte membranes increased after treatment with lectins or cadmium nitrate. Lectins possess biological activity modulated lipid peroxidation in membranes of erythrocytes from experimental animals, which depended on the time of adaptation. Differences were revealed in the effect of bacterial and plant lectins on lipid peroxidation in erythrocytes treated with the test xenobiotic.

Key Words: *lectins; erythrocytes; lipid peroxidation*

Biological membranes have numerous functions. Any dysfunction can be accompanied by changes in vital activity or death of the cell. Damage to the membrane lipid layer has the most severe consequences. Activity of membrane enzymes and receptors depends on some properties of the membrane lipid phase.

Lectins are extensively used in the studies of structural changes in the erythrocyte membrane upon exposure to adverse factors. Lectins are most informative molecular probes that allow us to identify and study changes in terminal carbohydrate residues under physiological and pathological conditions [2]. Moreover, lectins hold much promise as bioactive substances modulating properties of the cell membrane.

Binding of lectins to erythrocyte membrane is accompanied by various changes in its structure and stability, which depends on the type and concentration of these substances. For example, wheat-germ lectin is capable of stabilizing morphological characteristics of erythrocytes [9]. Lectin from *Pseudomonas aeruginosa* bacteria decreases osmotic resistance of erythrocytes and, therefore, destabilizes the membrane [8]. The interaction of concanavalin A with chicken erythrocytes is accompanied by a decrease in membrane viscosity [6]. These data suggest that binding of lectins to erythrocytes produces several reactions in the membrane or whole cell. However, the physiological role of most lectins remains unclear. Little is known about binding of lectins to erythrocytes under pathological conditions [6].

Here we studied the *in vitro* effects of fucose-specific lectins from bacteria and plants on the progression of structural and functional changes in erythrocyte membranes from experimental animals exposed to toxic stress.

MATERIALS AND METHODS

The heavy metal salt cadmium nitrate was used to produce toxic stress. Cytotoxicity of heavy metals is determined by several interrelated mechanisms. One of these mechanisms is intensification of lipid peroxidation (LPO). It should be emphasized that the inten-

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TABLE 1. Effect of Lectins on LPO in Rat Erythrocyte Membranes (*M*±*m*)

Parameter	Control	Lectin from *Azospirillum brasilense* Sp7	Lectin from golden chain tree
I_{max}	1.94±0.07 (100%)	2.23±0.11 (115%)	2.64±0.10 (136%)
S	10.31±0.41 (100%)	16.75±0.84 (145%)	13.78±0.55 (120%)

Note. Here and in Tables 2 and 3: percent change is shown in brackets.

sity of LPO increases in various diseases. Therefore, LPO is considered as a general pathological membrane process producing most severe damage to membranes of the plasma, mitochondria, and microsomes [3]. In our experiments the intensity of LPO was determined to evaluate the effect of lectins on erythrocytes.

Experiments were performed with the following fucose-specific lectins: bacterial lectin from *Azospirillum brasilense* Sp7; and plant lectin from golden chain tree (*Laburnum anagyroides*). The culture of azospirilla was grown on a liquid synthetic medium for flocculation at 37°C for 24 h [10].

Lectin was isolated from the cell surface as described elsewhere [7]. Bacterial lectin was purified by gel filtration [4]. We used commercial preparation of lectin from golden chain tree (Lektinotest).

Experiments were performed on female outbred albino rats weighing 250-300 g. The animals were divided into 4 groups (3 experimental groups and 1 control group). The toxic agent cadmium nitrate in a threshold daily dose of 2 mg/kg was administered intragastrically to rats of groups 1-3 for 10 days.

Ten animals from each group were decapitated 1 (group 1), 4 (group 2), and 8 days after the last toxic treatment (group 3). Control rats received intragastrically 1 ml physiological saline for 10 days. They were killed 1 day after the last administration of physiological saline.

The intensity of LPO was estimated by the biochemiluminescence method developed at the Institute of Biological Automation [1].

LPO was studied using washed erythrocytes from all animals taken before and after incubation with lectins from *Azospirillum brasilense* Sp7 and *Laburnum anagyroides*. The mixture of 0.5 ml erythrocyte suspension and 100 µl lectin (10 µg/ml) was incubated for 30 min. Chemiluminescence was measured after incubation of erythrocytes with lectin.

The maximum intensity of a rapid flash (I_{max}) reflected the presence of peroxidation products. The total yield of chemiluminescence (S) in erythrocyte membrane lipids characterized their oxidizability. The results were analyzed by Student's *t* test [5].

RESULTS

Preincubation of erythrocytes from control rats with lectins increased parameters of chemiluminescence (Table 1). Exposure of blood erythrocytes from control animals to bacterial lectin increased I_{max} and S by 15 and 45%, respectively.

Treatment of erythrocytes from control animals with plant lectin increased I_{max} and S (36 and 20%, respectively). These data suggest that both fucose-specific lectins increase the amount of peroxidation products and stimulate peroxidation in cells. Plant lectin was more potent in stimulating production of peroxidation products compared to bacterial lectin. Bacterial lectin more significantly increased the ability of lipids to undergo peroxidation.

We studied the effect of 10-day treatment with cadmium nitrate on LPO in erythrocyte membranes. The heavy metal salt was capable of modulating LPO (Table 2). I_{max} and S in group 1 rats increased by 51 and 30%, respectively. We showed that I_{max} and S increased by 73 and 89%, respectively, 4 days after the end of toxic treatment. On day 8, the value of S did not differ from the control.

We studied the effect of lectins on LPO in erythrocytes from animals receiving the toxic agent. The effect of lectins on erythrocyte membranes from ani-

TABLE 2. LPO in Rat Erythrocyte Membranes after 10-Day Treatment with Cadmium Nitrate (*M*±*m*)

Parameter	Control	Group 1	Group 2	Group 3
I_{max}	1.94±0.07 (100%)	2.94±0.15 (151%)	3.37±0.17 (173%)	2.18±0.11 (112%)
S	10.31±0.41 (100%)	13.46±0.54 (130%)	19.48±0.77 (189%)	10.22±0.41 (100%)

TABLE 3. Effect of Lectins on LPO in Rat Erythrocyte Membranes after Treatment with Potassium Nitrate (*M*±*m*)

Group	I_{max}	S	I_{max} ABL	S ABL	I_{max} LAL	S LAL
1	2.94±0.15 (100%)	13.46±0.54 (100%)	4.38±0.17 (148%)	20.17±0.80 (143%)	2.54±0.10 (86%)	10.37±0.41 (71%)
2	3.37±0.17 (100%)	19.48±0.77 (100%)	2.82±0.11 (83%)	19.13±0.76 (100%)	2.39±0.09 (71%)	12.91±0.51 (57%)
3	2.18±0.11 (100%)	10.22±0.41 (100%)	3.88±0.16 (178%)	21.78±0.86 (213%)	3.11±0.12 (142%)	28.66±0.14 (280%)

Note. ABL, bacterial lectin; LAL, plant lectin.

mals exposed to the toxic agent was compared to the control (Table 3). The results of experiments with erythrocyte membranes exposed only to the toxic agent served as the control.

After treatment with bacterial lectin I_{max} and S in group 1 rats increased by 48 and 43%, respectively. In group 2 animals I_{max} was below the control by 17%, while S practically did not differ from the control. I_{max} and S sharply increased in group 3 rats (by 78 and 113%, respectively).

The intensity of LPO in group 1 rats receiving plant lectin was below the control level. I_{max} and S in these animals decreased by 14 and 29%, respectively. The decrease in the test parameters was also observed in group 2 rats. I_{max} and S in these animals decreased by 29 and 43%, respectively, compared to the control. However, we revealed an increase in I_{max} and S in group 3 rats (by 42 and 180%, respectively). Activation of LPO in the lipid bilayer serves as a general mechanism underlying damage to cell membrane structures. Activation of LPO often leads to cell destruction, which does not depend on the nature of inductors. However, activation of the physiological antioxidant system and intensification of LPO are considered as a natural adaptive and compensatory process.

Our results indicate that LPO in erythrocyte membranes treated with the toxic agent is intensified 1 day after exposure to bacterial lectin. Parameters of LPO decrease to the control level after 4-day adaptation, but sharply increase on day 8 of the adaptation period.

As distinct from bacterial lectin, the intensity of LPO decreased 1 day after treatment with plant lectin. The test parameters progressively decreased over a 4-day adaptation period. However, we revealed significant activation of LPO on day 8 of adaptation (similarly to the effect of bacterial lectin).

These data indicate that biological activity of lectins contributes to modulation of LPO in animal cell membranes. We showed for the first time that the biological effect of lectins in animal cells depends not only on specificity, but also on the nature of these compounds.

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