CHANGES IN PHOSPHOLIPID-PHOSPHOLIPID RELATIONS AND ACTIVITY OF ANTIOXIDANT PROTECTION ENZYMES IN THE RAT LUNGS DURING DEHYDRATION

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A frequent disturbance of water and electrolyte metabolism is general dehydration, accompanied by changes in metabolism of the principal classes of substances: proteins, fats, and carbohydrates [1, 4, 5]. An important role in the development of adaptive and pathological reactions of the organism to dehydration is played by the lungs, for together with other organs (for example, the kidneys and skin), they participate in water excretion from the body. The role of the lungs in lipid metabolism also is very important. One cause of the disturbances found during dehydration may be a change in permeability of the membranes, due to a change in the content and properties of their phospholipids (PL). An important role in the modificaiton of membrane lipids is played by free-radical peroxidation, activation of which accompanied many pathological processes and is largely determined by the state of the enzyme systems of antioxidant protection.

The aim of this investigation was to study the content and composition of PL in the lungs during water deficiency, and also to examine the activity of enzymes detoxicating active forms of oxygen and lipid peroxides.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 180-200 g, divided into four groups, one of which served as the control. Dehydration was simulated by depriving the animals of water or liquid food for 3, 6, and 9 days. Extracts of lipids from lung homogenate were obtained by Folch's method. PL were fractionated in a thin layer of silica-gel in a system of solvents: chloroform–methanol–ammonia. The PL were identified with the aid of reference substances: phosphatidylcholine (PCh), phosphatidylserine (PS), phosphatidylethanolamine (PEA), sphingomyelin (SPM), and polyglycerophosphatides (PGP) (from Serva, West Germany) and by color tests. Lipid phosphorus was determiend by the method in [3]. Superoxide dismutase (SOD) activity was measured as inhibition of reduction of nitro-BT in a xanthine–xanthine oxidase system [7] on a Hitachi 557 (Japan) spectrophotometer. SOD from Calbiochem (USA) was used as the standard. Catalase activity was determined at 240 nm from the reduction in optical density of H_2O_2 using the test kits from Boehringer (West Germany). Glutathione peroxidase (GPO) activity was determined as oxidation of NADH in the reaction of reduction of oxidized glutathione [8]; glucose-6-phosphate dehydrogenase (G6P) activity was determined by measuring reduction of NADP at 340 nm using test kits from Boehringer. Total protein was determined by Lowry's method [10].

EXPERIMENTAL RESULTS

Analysis of the results showed a considerable decrease in the content of total PL in lung tissue during water deprivation, on account of a decrease in the content of nearly all fractions (Table 1). Only the concentration of lysophosphatidylcholine (LPCh) was increased on the 3rd and 6th days of the experiment. In normally functioning biological membranes particular importance is attached to constancy of the ratio between the phospholipid

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TABLE 1. Changes in Content and Spectrum of PL in Lungs (μmoles/g dry weight of tissue) during General Dehydration (M ± m)

Parameter	Control	Duration of dehydration, days		
		3	6	9
Glycerophosphate	6,55±0,78	4,64±0,58	3,26±0,41	2,67±0,41
Lysophosphatidylcholine	2,77±0,64	4,04±0,99*	3,78±0,76	1,33±0,41
PS	12,94±1,62	5,53±0,77*	8,34±1,44*	6,60±1,02*
SPM	33,20±1,93	26,35±1,24*	20,96±2,42*	26,36±1,61*
PCh	51,50±1,91	36,37±1,71*	30,51±1,67*	44,69±3,12
PEA	37,93±2,23	22,28±0,55*	17,91±0,80*	32,84±1,12
PGP	13,32±0,99	5,78±0,59*	6,01±0,69*	7,01±0,71*
Phosphatidic acids	5,99±0,69	4,04±0,65*	3,15±0,34*	2,73±0,69*
Total phosphorus	165,0±10,4	112,6±5,6*	95,2±3,2*	125,4±3,1*
$K_{npl/apl}$	3,89	5,77	4,18	6,43

Legend. *) Values differing significantly from control.

TABLE 2. Time Course of Changes in Activity of Antioxidative Enzymes in Rat Lungs during Water Deprivation (M ± m)

Parameter	Control	Duration of dehydration, days		
		3	6	9
SOD	0,101±0,005	0,204±0,029*	0,193±0,010*	0,260±0,016*
Catalase	3,51±0,32	4,62±0,78	5,71±0,81*	7,34±0,86*
GPO	65,71±6,01	65,20±4,28	85,70±7,90*	67,60±5,04
Glutathione reductase	24,26±1,63	25,92±1,93	24,92±1,57	20,56±1,65
G6P	33,12±2,36	47,68±3,77*	34,61±2,29	33,18±3,43

Legend. SOD and catalase activity expressed in activity units/mg protein and activity units/mg protein/min, respectively. GPO, glutathione reductase, and G6P activity expressed in nmoles NADPH/mg protein/min. *) Significant difference from control.

fractions and, in particular, the ratio between neutral and acid phospholipids (NPL and APL respectively). In the present experiments a relative decline of the APL was observed compared with NPL, so that $K_{npl/apl}$ was increased by about 1.5 times throughout the experiment. These changes in the phospholipid spectrum, in the opinion of some workers [2, 9], are evidence of activation of lipid peroxidation. Meanwhile, the oxidized lipids were accessible for the action of phospholipase, as was reflected in a fall in the concentration of PCh and a rise in that of LPCh. In turn, phospholipases disturb the structual organization of lipids and contribute to the intensification of their peroxidation by promoting access of oxygen.

Investigation of enzymes of antiperoxide protection in the rats' lungs during dehydration showed the presence of considerable changes in their activity (Table 2). SOD and catalase activity rose as dehydration worsened, to reach maximal values by the end of the experiment. This activation of catalase, synchronous with that of SOD, in the lung tissue facilitates destruction of hydrogen peroxide, inhibiting SOD activity. Disturbance of the metabolic function of the lungs was aggravated by disturbances of metabolism of lipids and biologically active substances. The catalytic ability of GPO also increased until the 6th day of dehydration, but by the 9th day it had fallen to the control level. On the 3rd day an increase in the rate of dehydration of G6P was observed, but at the remaining times the activity of this enzyme was unchanged. The increase in activity of the above-mentioned enzymes is probably compensatory in character in repsonse to intensification of lipid peroxidation.

Water deficiency in the body is thus accompanied by a disturbance of the phospholipid composition of lung tissue and by changes in activity of antioxidative enzymes, reflecting intensification of free-radical oxidation of lipids. These mechanisms may play a definite role in the development of pathological changes in the lung tissue and, in particular, in insufficiency of the surfactant system. This last hypothesis is confirmed by the presence of regions of dystelectasis and emphysema, and a reduction of aeration, revealed by histological investigation of dehydrated lungs [6].

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ACTION OF LASER RADIATION ON PEROXIDE CHEMILUMINESCENCE OF WOUND EXUDATE

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Low-energy radiation of helium-neon lasers with a wavelength of 632.8 nm has recently begun to be used in experimental and clinical practice. The stimulating action of laser radiation on regenerative processes in various organs and tissues has been demonstrated [1, 4, 5, 14]. Repair processes are accelerated by this factor, the area of the wound surface is quickly reduced, the development of granulation is accelerated, proliferation of connective tissue and epithelial cells is stimulated, phagocytosis is activated, and growth of microorganisms is slowed [3, 7].

We know that the principal role in the development of virtually every pathological process is played by lipid peroxidation (LPO), the state of which affects cellular metabolism, including the proliferative activity of cells [9, 12]. However, there is as yet no sufficiently simple and adequate method of estimating the effect of the LPO level on the rate of wound healing under the influence of laser irradiation. Recording chemiluminescence (CL) in the presence of hydrogen peroxide (H_2O_2), or peroxide CL, is a rapid and convenient method of monitoring the state of LPO and proteins in biological fluids [2, 13].

The aim of this investigation was to develop a method of recording peroxide CL of wound exudate and to study the effect of laser radiation on it.

EXPERIMENTAL METHOD

Experimental investigations of the exudate of wounds involving skin and fascia, healing by second intention, were undertaken.

The wound exudate for chemiluminescence analysis was taken during dressing by means of standard sterile filter paper disks 10 mm in diameter. The discs were applied to the wound surface and the exudate collected for 15 min. The disks were then placed in a flask

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