GLUTATHIONE AND ASCORBIC ACID METABOLISM AND ANTIOXIDATIVE ENZYME ACTIVITY IN TISSUES OF VITAMIN E-DEFICIENT RATS

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Vitamin E is an important factor in the system for protecting tissue against oxidative damage and the toxic action of xenobiotics. Data in the literature on changes in antioxidative enzyme activity and glutathione metabolism in vitamin E-deficient animals are incomplete and contradictory [4, 5, 8].

The aim of this investigation was to study the effect of alimentary vitamin E deficiency on the levels of reduced glutathione (GSH), glutathione disulfide (GSSG), cysteine, and ascorbic acid, and activity of glutathione-dependent enzymes, superoxide dismutase, and catalase in rat organs.

EXPERIMENTAL METHOD

Experiments were carried out on 77 male Wistar rats weighing initially 65-95 g. Vitamin E deficiency was induced by feeding the animals for 13-14 weeks on a semisynthetic diet, balanced with respect to all nutrients but deficient in vitamin E [3]. Control rats received food with the addition of 150 mg/kg of tocopherol acetate. The development of vitamin E deficiency was verified by determination of the degree of hemolysis of erythrocytes, and concentrations of free tocopherol in the blood plasma and the intensity of lipid peroxidation (LPO) in the liver [3]. Some animals for the last 2 weeks were transferred to a full diet (experiments to study of vitamin E deficiency).

After decapitation of the animals the liver, lungs, and heart were perfused with 0.154 M KCl solution and minced. Homogenates were kept in liquid nitrogen. After rapid thawing they were centrifuged at 800g for 10 min and used for the determination of superoxide dismutase (SOD), glutathione peroxidase (GP), glutathione reductase (GR), catalase (CT), formaldehyde dehydrogenase (FDH), gamma-glutamyl transferase (GGT), and glutathione-S-transferase (GT) activity with 1-chloro-2,4-dinitrobenzene (CDNB) and nitroglycerin (NG) as substrates. Methods of determination of enzyme activity and concentrations of GSH, GSSG, and cysteine in the tissue were described previously [1, 2]. The ascorbic acid concentration was determined by the reaction with 2,6-dichlorophenolindophenol [3].

EXPERIMENTAL RESULTS

Keeping the rats for 13-14 weeks on a diet deficient in vitamin E led to the development of avitaminosis E: the plasma tocopherol concentration was reduced, and hemolysis of erythrocytes and LPO in the liver were activated (Table 1). Transferring the animals to a complete balanced diet led to normalization of the tocopherol level and LPO activity and to a marked decrease in the degree of erythrocyte hemolysis.

The GSH concentration fell in the liver and lungs of the vitamin E-deficient animals. Conversely, the GSSG concentration rose, and cysteine remained unchanged. Correction of the vitamin E deficiency largely restored these changes to normal. The ascorbic acid concentration fell in the liver of the vitamin E-deficient animals, although its intake with the food

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TABLE 1. Concentrations (in μmoles/g) of GSH, GSSG, Cysteine, and Ascorbic Acid and Activity (in μmoles/min/mg protein) of Enzymes and LPO in Organs of Vitamin E-Deficient Rats (M ± m)

Parameter	Organ	Control (10-17)	Deficiency (11-21)	Deficiency + vitamin E (7-10)
Spontaneous hemolysis				
Plasma tocopherol, mg/liter	—	5.14±0.58	84.4±2.20*	12.5±0.89*
NADPH-dependent LPO	—	9.98±0.33	1.44±0.11*	11.8±0.98
GSH	Liver	23.1±1.28	100.6±6.71*	30.8±3.25
	»	7.23±0.15	5.17±0.16*	6.06±0.18*
	Lungs	2.57±0.082	1.57±0.054*	2.25±0.051*
GSSH	Liver	0.52±0.045	1.50±0.060*	1.06±0.086*
	Lungs	0.064±0.005	0.072±0.007	0.072±0.006
Cysteine	Liver	0.22±0.015	0.22±0.008	0.23±0.015
	Lungs	0.064±0.005	0.072±0.007	0.072±0.006
Ascorbic acid	Liver	1.50±0.068	1.05±0.049*	1.29±0.053*
GP	»	0.09±0.011	0.09±0.010	0.08±0.011
	Heart	0.10±0.003	0.07±0.006*	0.08±0.035
GR	Liver	0.068±0.007	0.049±0.004*	0.057±0.002
	Lungs	0.022±0.001	0.018±0.0008*	0.020±0.002
GT (substrate CDNB)	Liver	0.22±0.018	0.16±0.006*	0.19±0.009
	Lungs	0.029±0.002	0.021±0.001*	0.027±0.002
GT (substrate NG)	Heart	0.04±0.002	0.03±0.002*	0.04±0.003
FDH	Liver	0.016±0.0004	0.011±0.0004*	0.013±0.0007*
GGT	»	3.66±0.28	3.90±0.21	3.56±0.29
	Lungs	1.19±0.048	2.16±0.092*	1.58±0.0104*
CT	Liver	3.35±0.16	3.86±0.21	3.53±0.39
	Lungs	660±21	565±20*	665±52
SOD	Liver	18.2±1.7	23.8±1.9	20.2±2.0
	Heart	57.7±5.57	88.5±5.62*	58.2±4.58
		44.1±2.85	17.5±1.92*	36.0±2.61

Legend. *p < 0.05 Compared with control. PDH and GGT activity expressed in nanomoles min/mg protein, activity of NADPH-dependent LPO in nanomoles of malonic dialdehyde/30 min/mg protein. Number of experiments given in parentheses.

was the same as that of the control rats. We know that ascorbic acid and GSH, like tocopherol, can interact with active forms of oxygen and with free radicals, reducing them [6]. For that reason the decrease in the concentrations of these substances in tissues of vitamin E-deficient animals was attributable to their increased utilization to make good the tocopherol deficiency. Accumulation of GSSG in the liver was probably connected with a disturbance of its regeneration to GSH on account of a fall in GR activity. GP activity in the liver of the vitamin E-deficient rats was essentially unchanged, and only in the heart was it reduced a little. GT activity in the tissues of the animals was depressed whereas GGT activity in the liver was increased. Since GGT is involved in the working of the gamma-glutamyl cycle (containing synthesis and breakdown of glutathione), it can be tentatively suggested that the glutathione turnover is intensified in vitamin E deficiency.

CT activity in the liver of the vitamin E-deficient rats was somewhat depressed, possibly due to the property of vitamin E of regulating heme synthesis [7]. SOD activity in the liver was considerably increased, probably as a compensatory reaction of diminution of the antioxidant role of vitamin E.

Thus in vitamin E deficiency there is an adaptive increase in the intensity of glutathione and ascorbic acid ultiation, and increased activity of SOD and GGT, whereas activity of the glutathione-dependent enzymes and of CT falls. This points to the selective character of response of the antioxidative system to a deficiency of one of its factors (vitamin E). Relations between the individual factors of the antioxidative system are evidently quite complex.

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ACTIVITY OF ENZYMES OF ADENOSINE METABOLISM IN HUMAN NATURAL KILLER (NK) CELLS DURING ACTIVATION AND INHIBITION OF THEIR CYTOTOXIC ACTIVITY

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Experiments in vivo and in vitro have shown that the principal modulators of the cytotoxic activity (CTA) of natural killer (NK) cells are interferon (IF) and prostaglandins of various types, mainly of type E [4, 7]. IF and certain interferonogens induce differentiation of NK cells and increase the rate of killing, so that the CTA of these cells is quickly and essentially increased [7, 12]. Prostaglandins of type E (PGE), which are locally active short-living hormones, exhibit different biological activity depending on their concentration [4, 6]. However, the concrete biochemical mechanisms determining CTA of NK cells have hardly been studied at all. It has been shown that an important role in the functioning and differentiation of immunocompetent cells is played by enzymes, namely adenosine deaminase (ADA; EC 3.5.4.4) and 5'-nucleotidase (5-N; EC 3.1.3.5), which regulate the intracellular adenosine concentration [11].

The aim of this investigation was to study activity of ADA and 5-N in human NK cells when treated in vitro with the IF inducer – Newcastle disease virus (NDV) or a preparation of PGE_2.

EXPERIMENTAL METHOD

Human NK cells were isolated from packed lymphocytes obtained on an "Amicon" blood separator (USA) at the bone marrow bank of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, by the method described previously [2] in a Ficoll–Verografin density gradient, followed by purification and concentration of NK cells in a Percoll stepwise density gradient [3]. CTA of the NK cells was studied in the cytotoxic test (CTT) with target cells consisting of human lymphoma MOLT-4 cells labeled with ^{51}Cr [3]. The percentage lysis of MOLT-4 target cells was determined with ratios of effector to target cells of 50:1 and 25:1. The isolated NK cells were treated for 15 min with NDV at 20°C. After the end of treatment the NK cells were washed 3 times with medium RPMI-1640, containing 10% of bovine serum. A commercial preparation of PGE_2 (Sigma, USA) was diluted in medium RPMI-1640 and added (in a concentration of 10^{-5} to 10^{-12} M) to the test NK cells. After incubation for 30 min at 37°C the NK cells were sedimented by centrifugation and their CTA was investigated in the CTT. In parallel tests, some cells obtained after treatment with MDV and PGE_2 were lyzed in a solution containing 20 mM Tris-HCl, 5 mM $MgSO_4$, pH 7.4, and activity of ADA and 5-N was determined in the lysate with the aid of (8-^{14}C)-adenosine and (8-^{14}C)-adenosine monophosphate by ascending paper chromatography [1] in a micromodification. ADA activity was expressed in nanomoles of inosine and hypoxanthine per minute per 10^8 cells, and

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