CONCENTRATIONS OF LIPID PEROXIDATION PRODUCTS AND ANTIOXIDANT ENZYME ACTIVITY IN MYOCARDIUM AND LIVER OF RATS DIFFERING IN VITAMIN E INTAKE

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Intensification of lipid peroxidation (LPO) in biomembranes leads to a disturbance of normal cell function [4]. LPO in the body is regulated by protective systems, including natural antioxidants [2], and antioxidative enzymes utilizing active forms of oxygen (superoxide dismutase — SOD) and lipoperoxides (glutathione peroxidase — GP) [4, 10].

The best known natural antioxidant α-tocopherol (TP), is not synthesized in animal cells, and accordingly the supply of this vitamin to the body depends primarily on alimentary factors [13]. It has been shown that vitamin E deficiency causes the development of certain functional and structural disturbances of the motor apparatus, the regenerative system, the myocardium, and so on [1, 11, 13]. It has been shown to be possible, in principle, to modify activity of antioxidative enzymes by administering TP [6]. Nevertheless, data in the literature on changes in activity of the protective enzymes following variation of the vitamin E level in the tissues are contradictory and, moreover, the investigations themselves are insufficiently systematic.

Since the elucidation of relations between different components of the antioxidative system of the cell is of practical as well as theoretical importance, and also with the more widespread use of antioxidants for prophylactic or therapeutic purposes, it was decided to study changes in concentrations of LPO products and activity of antioxidative enzymes in the myocardium and liver of rats differing in their vitamin E intake.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing initially 60-80 g, receiving a semisynthetic diet for 2 months differing in its content of DL-α-tocopheryl acetate [7]. The animals were divided into three groups: 1) rats with a normal supply to TP (100 mg/kg diet); 2) rats with a reduced TP content in the diet (6 mg/kg diet); 3) rats receiving a diet without TP. The TP concentration in the tissues was determined spectrofluorometrically [14] and concentrations of primary LPO products (acyl hydroperoxides) in lipid extracts were determined on the basis of the characteristic absorption of conjugated dienes at 232 nm on a "Hitachi-557" spectrophotometer (Japan) [15]. Concentrations of secondary LPO products, namely intermolecular cross-linkages of amine-containing phospholipids of the Schiff base type—were determined spectrofluorometrically in lipid extracts on a "Hitachi MPF-4" fluorometer [9]. The concentration of malonic dialdehyde, a product of oxidative destruction of lipoperoxides, in the tissue homogenates was recorded by the reaction with 2-thiobarbituric acid [12]. Activity of the antioxidative enzymes SOD and GP in the tissue homogenates (850 g × 10 min supernatant) was determined as described previously [5].

EXPERIMENTAL RESULTS

The results indicate a sharp decrease in the TP concentration in the liver and heart of rats receiving a semisynthetic diet with a low vitamin E level (6 mg/kg) or a diet without

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TABLE 1. Concentrations of TP and LPO Products and Activity of Antioxidative Enzymes in Tissues of Rats (numerator — in the heart, denominator — in the liver) with Avitaminosis E (M ± m, n = 6)

Parameter	Group of animals		
	1	2	3
TP, μg/g tissue	50,2±6,1 / 67,7±2,9	17,0±2,1* / 30,5±2,5*	13,1±0,5* / 25,4±2,2*
Acyl hydroperoxides (A_{232}/ A_{208} × 100)	25,7±2,1 / 38,4±3,8	24,2±4,1 / 32,1±6,0	34,2±2,4* / 34,2±4,2
Malonic dialdehyde, μmoles/g tissue	0,19±0,02 / 0,27±0,03	0,25±0,03 / 0,30±0,	0,32±0,03* / 0,34±0,03
Flourescent products, relative units/g of lipids	1,00±0,21 / 2,52±0,30	1,50±0,22 / 2,24±0,26	1,57±0,54 / 2,41±0,48
SOD, units/mg protein	45,3±2,2 / 64,6±7,0	42,8±1,5 / 65,3±5,9	33,6±1,7* / 76,3±5,5
GP, units/g protein	114,5±5,7 / 81,5±4,6	120,0±5,6 / 80,0±2,8	99,0±9,7 / 74,5±7,9

vitamin E (Table 1). After 2 months on a diet with the addition of 6 mg DL-α-tocopheryl acetate/kg diet, the TF concentration in the liver and heart of the rats was reduced by 2.2 and nearly 3 times respectively. However, neither in the liver nor in the heart of the animals of this group were any changes observed in the concentration of primary and secondary LPO products, and also in activity of the antioxidative enzymes studied compared with the tissues of animals receiving the semisynthetic diet with the addition of 100 mg/kg of vitamin E. Evidently the level of supply of vitamin E to the rat ensured by its concentration of 6 mg/kg in the diet is sufficient to maintain the antioxidative defense of the body at the necessary level.

The TP concentration in the liver and heart of the animals with avitaminosis E was reduced by 2.7 and 3.8 times respectively compared with the control group (Table 1). The concentrations of primary (acyl hydroperoxides) and secondary (malonic dialdehyde) LPO products in the heart of rats with avitaminosis E was 1.3 and 1.7 times higher respectively than that in the heart of the control group of rats. Meanwhile, a reduction of SOD activity by 1.3 times was observed in the heart of the animals with avitaminosis E compared with that in animals receiving an adequate amount of TP (control). The concentration of LPO products and activity of the enzymes of antioxidative defense in the liver of the animals with avitaminosis E were unchanged compared with the control.

Thus both a subnormal level and the total absence of vitamin E in the diet under these experimental conditions did not lead to any significant changes in the concentrations of LPO products or the activity of antioxidative enzymes in the liver. In advanced stages of vitamin E deficiency, LPO products were observed to accumulate in the heart and SOD activity was reduced.

The absence of intensification of LPO in the liver of rats with avitaminosis E, when the level of activity of antioxidative enzymes remained unchanged, may be indirect evidence of the important role of the defensive enzyme systems of the body in the regulation of free-radical processes. It must also be recalled that under normal conditions the liver is supplied with excessive stocks of TP [8], so that a threefold reduction in its concentration may be insufficient to activate LPO (Table 1). The results of this investigation indicate that the myocardium is more sensitive than the liver to alimentary vitamin E deficiency. These facts explain the results of investigations in which disturbances of cardiac function were found in avitaminosis E [1, 3, 11], and it may accordingly be concluded that deficiency of natural antioxidants is not always compensated by activation of the defensive enzymes. The results of this investigation also indicate that LPO in the myocardium can be corrected with the aid of TP.

LITERATURE CITED

1. Yu. V. Arkhipenko, L. M. Dzhaparidze, D. V. Gutkin, et al., Vopr. Med. Khimii, No. 1, 122 (1987).

2. E. B. Burlakova, Kardiologiya, No. 8, 48 (1980).
3. L. M. Dzhaparidze, L. M. Belkina, R. S. Dosmagambetova, et al., Vopr. Pitan., No. 3, 25 (1986).
4. V. Z. Lankin, Biochemistry of Lipids and Their Role in Metabolism [in Russian], Moscow (1981).
5. V. Z. Lankin, D. B. Bandyshev, A. K. Tikhaze, et al., Dokl. Akad. Nauk SSSR, 259, No. 1, 229 (1981).
6. V. Z. Lankin, A. K. Tikhaze, D. R. Rakita, et al., Ter. Arkh., No. 5, 58 (1985).
7. V. B. Spirichev, L. M. Bronshtein, and I. I. Matusis, Experimental Vitaminology [in Russian], Minsk (1979), pp. 18-57.
8. V. A. Shaternikov, Vitamins [in Russian], Moscow (1974), pp. 125-150.
9. W. K. Bidlack and A. L. Tappel, Lipids, 8, 203 (1973).
10. L. Flohe, Free Rad. Biol., 5, 223 (1982).
11. H. S. Lee and A. S. Csallani, Lipids, 22, 104 (1987).
12. H. Ohkawa, N. Ohishi, and K. Agi, Anal. Biochem., 95, 351 (1979).
13. M. L. Scott, The Fat-Soluble Vitamins, New York (1978).
14. S. V. Taylor, M. P. Lambdem, and A. L. Tappel, Lipids, 11, 530 (1976).
15. C. G. Villard and A. L. Tappel, Lipids, 8, 183 (1973).

LOWERING THE STIMULATED FREE INTRACELLULAR CALCIUM LEVEL IN PLATELETS IN THE REFRACTORY STATE

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ADP is a specific activator of platelets which can induce platelet aggregation both in vitro and in vivo. Preincubation of platelets with ADP lowers their sensitivity to the secondary action of this inducer or, as is usually stated, transition of the platelets into a state of refractoriness [5]. Reduction of aggregation in the refractory state cannot be explained entirely by the formation of aggregation inhibitors, namely adenosine and AMP [8].

On the other hand, we know that platelet activation depends essentially on synthesis and (or) movement of secondary messengers, such as calcium ions, inositol triphosphate, diacylglycerol, and cAMP [6]. The connection between refractoriness and the secondary messenger system has not been adequately studied. Previously the writers found changes in the concentration of calcium ions bound with the platelet membrane corresponding to the state of refractoriness [2]. The aim of this investigation was to study changes in the free Ca^{++} concentration in the cytoplasm during a change in shape and aggregation of platelets in the normal and refractory state.

EXPERIMENTAL METHOD

The following preparations were used: Indo 1-AM, Indo 1, and MOPS from "Calbiochem," apyrase, ADP, and bovine serum albumin from "Sigma," and EGTA from "Fluka." All other reagents were of the chemically pure grade.

A rabbit's blood was stabilized with standard acid citrate–dextrose solution in the ratio of 8.5:1.5. Platelets were washed by the method in [3]. MOPS–NaOH was used as the buffer. After centrifugation of the platelet plasma and removal of the supernatant the platelets were resuspended in 1% Tyrode solution, incubated with 10 μM Indo 1-AM for 30-45 min at 37°C, and washed twice.

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