Macrophages of Subcutaneous Connective Tissue after Treatment with α-Tocopherol and Dehydration

L. G. Proshina

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Morphological aspects of macrophage restructuring in rat subcutaneous connective tissue after treatment with α-tocopherol and alimentary dehydration are studied. The antioxidant did not cancel the response of macrophages to changes in water-salt homeostasis (increased number, ultrastructural changes, or metabolic activity), but modified it by decreasing the effect of dehydration stress.

Key Words: *macrophage; subcutaneous connective tissue; α-tocopherol*

Redox homeostasis is normally maintained in cells and tissues of live organisms. Excessive production of lipid peroxides (LPO) under the effect of exogenous and endogenous factors leads to "oxidative stress". Dehydration caused by ecological, experimental, or pathological factors can be regarded as a stress situation leading to LPO activation [1]. High polyfunctional activity of macrophages and the presence of peroxidase, superoxide dismutase, catalase, and glutathione-reducing systems in these cells allows no doubt of their participation in utilization of lipid peroxides in health and emergency [3,4,8,9]. Tissue protection from free-radical oxidation is determined by the status of its antiperoxide enzymatic systems and the set of antioxidants. Therefore, antioxidants can be used to modify morphofunctional activity of macrophages for increasing the resistance of an organism to dehydration.

Our purpose was to study morphology and functional activity of subcutaneous connective tissue macrophages during treatment with exogenous α-tocopherol (α-TP) by comparing the count and composition of the population, analysis of the hydrolytic and redox enzymes, morphometric assessment of their cytoplasm ultrastructure, and evaluation of the LPO status.

Department of Histology and Embryology, Russian State University of Peoples' Friendship, Moscow; Department of Histology and Embryology, Medical Institute, Karaganda

MATERIALS AND METHODS

Outbred male albino rats weighing 180-200 g were used. Intact animals were fed standard diets with free drinking. Dehydration was induced by feeding dried fodder without access to water and urine for 3, 6, and 9 days. The antioxidant status was increased by oral α-TP (Serva) in a dose of 50 mg/kg. Administration of α-TP was started 6 days before the beginning of water deprivation and went on during the entire dehydration period. Cell population of subcutaneous connective tissue was studied on film preparations stained with Weigert's iron hematoxylin. Metabolic activity of macrophages and their contribution to lipid metabolism were assessed using a complex of cytochemical methods for measuring the activities of redox and hydrolytic enzymes: β-hydroxybutyrate dehydrogenase, lactate dehydrogenase, α-glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenase, NAD-diaphorase, nonspecific esterase, and acid phosphatase [2]. Enzyme activities were evaluated by optic density of the cytoplasm under a Lumam I-2 microscope and expressed in arbitrary units. LPO activity in blood plasma was assessed from the content of dienic conjugates (DC) [5] and malonic dialdehyde (MDA) [10]. For study of macrophagal ultrastructure, fresh-cut fragments of subcutaneous connective tissue were fixed in 2.5% glutar aldehyde and then in 1.5% osmium tetroxide

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in phosphate buffer and embedded in Araldite. The sections were contrasted with lead citrate and examined under a JEM-100B electron microscope. Morphometric study was carried out according to basic rules of stereology [6]. The instrument for measurements was a regular test grid with a 1-cm step. The volume (in comparison with the cytoplasm) of the main cell components was measured: mitochondria, primary lysosomes, vacuoles with contents of low electron density, phagosomes, granular endoplasmatic reticulum, and lipid incorporations. The mean size of these organelles and their number per unit of cytoplasm volume were estimated. A total of 100 cells were compared. The status of peripheral blood macrophages was monitored by differential blood count. Smears were methanol-fixed and stained according to the Romanovskii-Giemsa method. Osmotic concentration of blood plasma was measured by kryoscopy (OMKA I Ts-01 osmometer). The significance of differences was evaluated by Student's t test at 95% confidence probability ($p<0.05$).

RESULTS

The proportion of macrophages among other cells in subcutaneous connective tissue is 25.4%. Relative content of macrophages per 1000 fibroblasts is 352± 8. Metabolic activity of macrophages is higher than of other cells in subcutaneous connective tissue (β-hydroxybutyrate dehydrogenase activity is 3 times higher than in fibroblasts). The population of macrophages is heterogeneous by enzyme activities: there are cells with high, medium, and low activities of hydrolytic and oxidative enzymes. The heterogeneity of the population has been confirmed by others [3,4, 7]. Analysis of ultrastructure of the cytoplasm showed sufficient saturation with organelles (Fig. 1). Total volume of all organelles and vesicles is 22.41±1.82 of total cytoplasm volume. Experimental dehydration leads to a rapid loss of body weight (by 22-28%), mainly due to a decrease in the fatty tissue content. Concentration of the plasma increases by 20% by the 9th day of water deprivation. The plasma osmolarity increase is paralleled by increase in the concentration of toxic lipid peroxides, with the maximum increase (by 130%) in dienic conjugates on day 3 ($p<0.05$) and in MDA on day 6 (by 120%, $p<0.05$). The macrophage population increases and then decreases in dehydration. The relative content of macrophages reaches the maximum on day 6 of dehydration (652± 6) and decreases on day 9 (508±8). The increment in macrophage count may be due to young precursor cells, which is proven by increased count of peripheral blood monocytes. On day 3 of dehydration, the count of monocytes increases from 5.2% (intact control) to 10.9% ($p<0.05$). Cytoplasmatic enzyme activities are diversely changing during dehydration. Activities of many enzymes increase on day 3 of water deprivation (Table 1).

Increased activity of acid phosphatase may indicate labilization of lysosomal membranes and correlates with accumulation of LPO products (dienic conjugates and MDA). On day 6, the activities of virtually all studied enzymes decrease, except acid phosphatase, whose activity decreases on day 9. Ultrastructure of macrophages undergoes characteristic changes in dehydration (Fig. 2). The volume of vesicles decreases from 22.41±1.82 (in intact animals) to

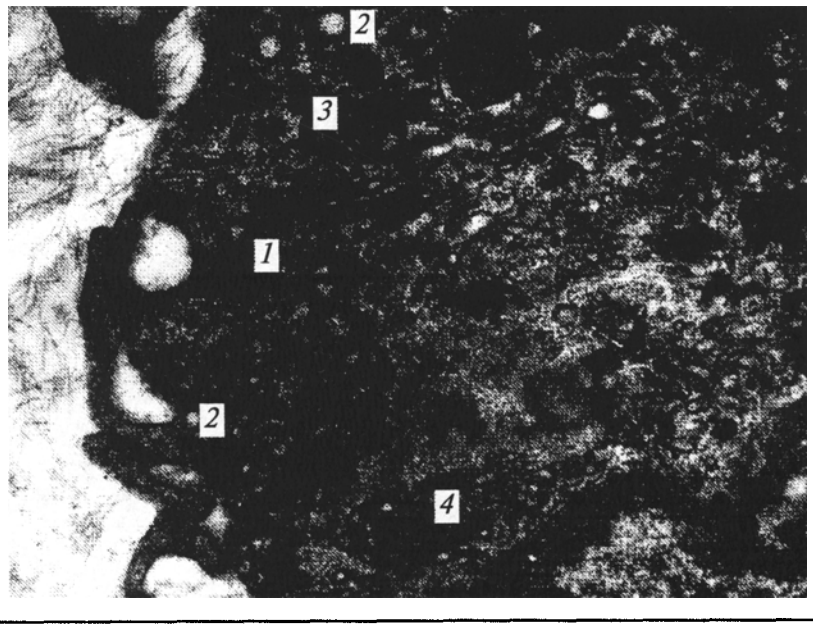

Fig. 1. Fragment of a macrophage in subcutaneous connective tissue of intact rats, ×22,000. *1*) lysosome; *2*) vesicles; *3*) mitochondrion; *4*) Golgi complex.

Table 1. Activities of Enzymes in Connective Tissue Macrophages of Albino Rats Exposed to Dehydration and α-TP Treatment, arb. U/probe area (*M±m*)

Day and conditions of experiment (*n*=250)	Enzymes						
	β-hydroxybutyrate dehydrogenase	nonspecific esterase	lactate dehydrogenase	glucose-6-phosphate dehydrogenase	α-glycerophosphate dehydrogenase	NAD-diaphorase	acid phosphatase
Intact	23.6±0.5	37.9±0.5	32.5±0.8	28.6±0.8	40.1±0.8	30.6±0.5	18.6±0.5
Dehydration, day 3	28.6±0.8*	46.3±0.8*	36.5±0.9*	33.3±0.6*	42.5±0.8*	33.3±0.8*	40.3±0.6*
Dehydration, day 3+α-TP	30.2±0.6*+	48.5±0.5*+	36.6±0.6*	36.2±0.7*+	46.4±0.6*+	36.2±0.6*+	29.0±0.6*+
Dehydration, day 6	17.9±0.5*	35.4±0.6*	28.4±0.7*	26.3±0.6*	32.5±0.6*	28.1±0.7*	28.9±0.5*
Dehydration, day 6+α-TP	28.0±0.6*+	45.7±0.5*+	30.1±0.6*	34.2±0.5*+	43.6±0.5*+	32.7±0.5*+	20.9±0.7*+
Dehydration, day 9	16.3±0.7*	21.6±0.7*	27.5±0.5*	22.4±0.7*	28.6±0.7*	25.3±0.8*	14.3±0.5*
Dehydration, day 9+α-TP	18.2±0.6*+	34.0±0.6*+	28.7±0.9*	25.7±0.8*+	30.9±0.6*+	28.2±0.6*+	16.9±0.6*+

Note. $p<0.05$ *vs. intact rats, +vs. rats with dehydration without α-TP.

16.80±1.71 (on day 9 of dehydration). Phagocytic activity increases (increased number of phagosomes) and lysosomes are formed (the number of small primary lysosomes increases). In parallel, the number of electron-transparent vacuoles in cell decreases. The volume of lipid incorporations increases: the number of cell profiles containing lipid droplets increases to 43% on day 9 of experiment (*vs.* 3% in intact animals).

Dehydration during α-TP treatment caused a more intense increase in the number of macrophages as soon as on day 3 of experiment. The maximum increase in the number of cells was observed on day 6, although macrophagal reaction was weaker than in dehydration alone. On day 9 of water deprivation, a stronger macrophagal response was observed than in the group without α-TP pretreatment. The increase in the count of macrophages correlated with the decrease in their size. These results indicate decreased production of toxic LPO metabolites (dienic conjugates and MDA) and lowering of plasma osmotic concentration. Cytochemical activities of virtually all enzymes tested, except acid phosphatase, were higher after dehydration in parallel with α-TP treatment than in the same terms of dehydration alone. The activity of acid phosphatase decreased on day 9, but remained higher than in animals administered no α-TP. These findings can be interpreted as an improvement of lysosomal membrane stability. Cell destruction was often observed on day 9 of dehydra-

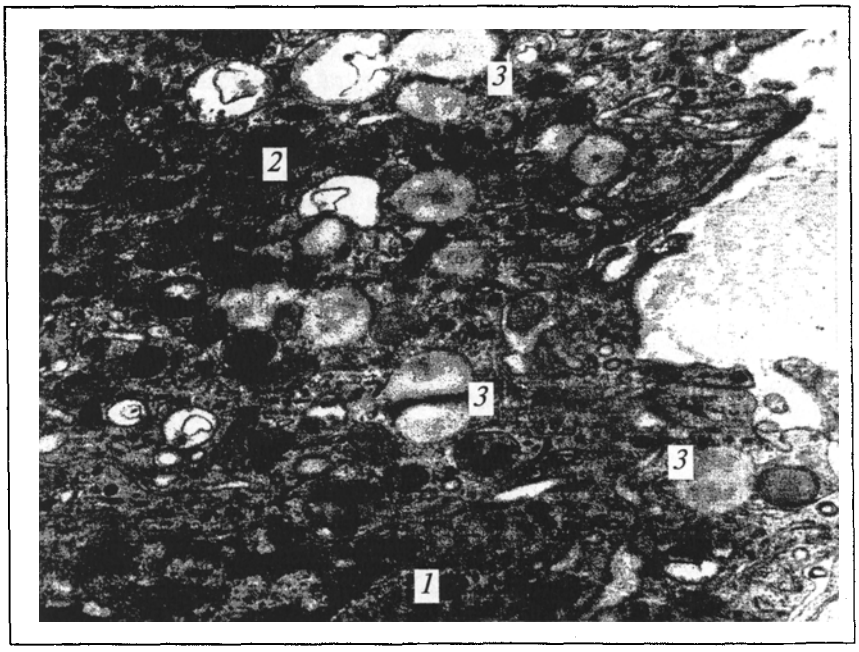

Fig. 2. Fragment of a macrophage in subcutaneous connective tissue of rats subjected to dehydration, ×17,500. *1*) lysosomes; 2) mitochondrion; *3*) lipid incorporations.

Fig. 3. Fragment of a macrophage in subcutaneous connective tissue of rats subjected to dehydration during α-tocopherol treatment, ×17,500. *1*) lysosomes; 2) mitochondrion.

tion without α-TP saturation, which can be regarded as a failure of compensatory potential of macrophages. In animals pretreated with α-TP, ultrastructural changes in dehydration (Fig. 3) correlate with changes in metabolic activity and indicate a lower strain for the organism. Dehydration after pretreatment with α-TP brings about an increase in the volume of mitochondria, hypertrophic organelles appear, and relative surface of granular endoplasmatic reticulum and Golgi complex increase. Total volume of organelles increases to 22.71±1.15 on day 3, while on day 6 it is 22.45±1.42 ($p<0.05$). Cell infiltration with lipids decreases, which can be due to prevention and decrease of lipid modification. Cell profiles contain more electron-transparent vacuoles and less phagosomes. Therefore, α-TP exerts a protective effect under conditions of dehydration stress involving LPO activation. A-TP stimulated the compensatory potential of cells and organism in general (mortality dropped by 52%). α-TP did not abolish the effect of dehydration but increased the adaptive compensatory potential and resistance to it.

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