EFFECT OF TOCOPHEROL ON FUNCTIONAL RESERVES OF PHAGOCYTES

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It is well known that tocopherol (TP) can protect the cell functions in many pathological processes accompanied by inflammation, intensification of lipid peroxidation (LPO) and cell damage. The mechanisms of the antiinflammatory and antiatherogenic action of TP include its ability to inhibit LPO and to give rise to characteristic structural changes in biological membranes, leading to the intensification or depression of certain cell functions. At the organism level these effects of TP are realized in complex systemic reactions and they determine an increase in resistance of the body to various harmful factors [3]. An important step in the understanding of the universal therapeutic effect of TP, in our view, must be the investigation of its influence on the effector cells of inflammation and, on the system of mononuclear phagocytes (MPS) as a whole, for it has the key role in the maintainence of nonspecific resistance of the body.

The aim of this investigation was to study correlation between TP accumulation in the membranes, changes in their viscosity, and the functional activity of phagocytes, namely their nonspecific bactericidal activity, and also the ability of the macrophages to esterify cholesterol entering the cell in the composition of modified low-density lipoproteins (acyl-LDL), which may serve as a criterion of resistance of the body to atherosclerosis [10].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 200-240 g (seven animals in each group). In addition to the standard diet, the experimental rats were given α-tocopherol acetate in a dose of 4 mg in 30% oily solution daily for 7 days; the control animals received the equivalent amount of sunflower oil [6]. Resident macrophages (Mph) were obtained from the peritoneal fluid [8] under ether anesthesia, by injecting 25 ml of medium A: medium RPMI-1640 (from "Serva," West Germany), gentamicin, and penicillin (100 U/ml). The peritoneal cells were harvested by centrifugation (400 g, 10 min) and resuspended in medium A containing 10% rat serum, up to a density of (2-3) $\cdot 10^6$ cells/ml. The suspension was introduced in a volume of 2 ml into plastic Petri dishes 35 mm in diameter ("Falcon") and incubated at 37°C for 2 h. The cell monolayer was washed seven times with 3 ml of medium A and the Mph were used in the experiments. Their content of TP was studied as in [11] and LPO products (conjugated dienes) were determined from the UV spectra of the lipid extract [1]; the viscosity of the membrane lipids was studied by means of a pair of fluorescent probes (pyrene and the pyridine derivative OSP-14 [2]. The bactericidal activity of Mph, both spontaneous and induced by addition of zymosan granules to the incubation medium (50 particles per cell for 1 h before the investigation) — by the nitro-BT test: the number of cells around which diformazan was deposited per 100 Mph was counted [2]. The rate of synthesis of cholesterol esters in Mph was determined from incorporation of (1-^{14}C)-oleate into lipids [9] in the presence and in the absence of acetylated lipoproteins. For this purpose Mph were cultured in medium A containing 0.2% bovine serum albumin (BSA) and 0.2 mmole (1-^{14}C)-oleate, bound with BSA, for 18 h in an atmosphere of CO_2 (5%) and air (95%). The cell monolayer was washed twice with 2% BSA solution in medium 199 and 5 times with medium 199. LDL (1.019-1.055 g/ml) were

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TABLE 1. Concentrations of TP and LPO Products, Viscosity of Membrane Lipids of Mph, and Their Spontaneous and Zymosan Granule-Induced Bactericidal Activity (M ± m)

Experimental conditions	TP, μg/ml protein	Conjugated dienes, E_{233}/mg lipids	Viscosity of membrane lipids, P	Number of cells reducing nitro-BT, %	Activation index of Mph
Control	4,3±0,21	1,1±0,18	1,2±0,01	12,4±3	2,7±0,2
TP	5,3±0,18*	1,5±0,20	0,8±0,03**	7,9±2,7	4,8±0,3**

Legend. * $p < 0.05$ compared with control.

TABLE 2. Incorporation of (1-^{14}C)-Oleate into Cholesterol Esters of Mph of Rats in the Presence of Acetylated LDL (50 μg protein/ml medium 199) (M ± m)

Experimental conditions	Incorporation of oleate into cholesterol esters, nmoles/mg cell protein during incubation for 18 h
Control	16±0,9
Control + acetylated LDL	198±3,6 ($p_{1-2}<0,001$)
TP	19±1,8 ($p_{3-4}<0,001$)
TP + acetylated LDL	460±5,7 ($p_{2-4}<0,05$)

isolated from the donors' plasma by ultracentrifugation [4]. Acetylation of LDL was carried out with the aid of acetic anhydride [7]. Binding of (^{14}C)-oleate ("Amersham," England) with specific radioactivity of 56 mCi/mmole, with BSA free from fatty acids, was carried out by adding a solution of labeled oleic acid in 30 μl acetone to a 10% solution of BSA in 0.05 M phosphate buffer, pH 7.5, in 0.15 M NaCl [9]. The solution was aerated with nitrogen at 37°C until the smell of acetone disappeared.

Specific radioactivity of the bound (1-^{14}C)-oleate was 4200-8600 cpm/nmole BSA.

EXPERIMENTAL RESULTS

Addition of TP to the diet led to a twofold increase in its concentration in the blood plasma (from 6 ± 0.2 to 12 ± 0.7 μg/ml, $p < 0.001$). Its concentration in Mph rose by 30% (Table 1). Changes in the concentration of LPO products and conjugated dienes were not observed, but the viscosity of the membrane lipids of Mph of rats receiving TP was reduced. Reduction of viscosity can be regarded as a characteristic feature of increased activity of the phagocytes [5]. The study of the bactericidal activity of Mph showed that in the experimental cells it may even have a tendency to fall compared with the controls (Table 1). However, the Mph of the experimental rats responded to stimulation by zymosan granules by a much greater increase in their activity.

It will be clear from the results given in Table 2 that during culture of Mph in medium without acetyl-LDL the rate of incorporation of (1-^{14}C)-oleate into cholesterol esters did not differ in Mph of control rats and those receiving TP. On the addition of acetyl-LDL to the incubation medium (50 μg protein/ml medium) the rate of esterification of cholesterol in the control Mph was increased 12-fold, but in the experimental Mph 24-fold, evidence of increased functional capacity of the Mph of rats receiving TP, to utilize modified LDL.

Thus Mph of rats receiving TP differed from the controls in their content of TP and the reduced viscosity of their membrane lipids. The experimental cells responded to presentation of stimuli (zymosan granules and acetyl-LDL) by a significantly greater increase in activity: bactericidal, connected with production of active forms of oxygen, and metabolic, connected with elimination of modified LDL. It can be concluded from these results that under the influence of TP the functional reserves of Mph are increased. It can be tentatively suggested that the successful use of TP in the treatment of atherosclerosis and of other widely different diseases of inflammatory nature may be connected with increased reactivity of the effector cells of inflammation, and possibly of the MPS as a whole.

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REGULATION OF SUPEROXIDE DISMUTASE ACTIVITY DURING DEEP HYPOTHERMIA BY SIMULTANEOUS ADMINISTRATION OF WATER- AND LIPID-SOLUBLE ANTIOXIDANTS

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In connection with the use of an assisted circulation (AC) in contemporary heart surgery deep hypothermia (DH) of the myocardium is frequently carried out. The use of DH enables the vessels of the heart to be occluded for a comparatively long time during an operation. DH reduces the consumption of oxygen and the energy reserves, but it also disturbs the coordination of metabolism [7, 8, 10]. The leading causes of this are increased secretion of catecholamines during hypothermia [5] and subsequent activation of free radical generation and of lipid peroxidation [10]. Restoration of perfusion potentiates the action of these aggressive factors, and this may be the cause of unfavorable results of operations on the heart. Protection of the heart under both normal and extremal conditions is effected by enzymic and nonenzymic antioxidative systems. Under conditions of DH the heart is protected by maintenance of activity predominantly of superoxide dismutase (SOD) [1-3, 9].

In this investigation the action of panthetin and α-tocopherol was studied on activity of free radical generation during DH in the myocardium and blood.

EXPERIMENTAL METHOD

SOD activity and secretion of adrenalin and noradrenalin were investigated during cooling and long-term DH lasting from 30 to 60 min, at intervals of 10 min. The work was done on male Wistar rats weighing 250-270 g. The animals

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