Effect of Ubiquinone Q_{10} and Antioxidant Vitamins on Free Radical Oxidation of Phospholipids in Biological Membranes of Rat Liver

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We studied the effects of 30-day peroral treatment with β-carotene, a complex of antioxidant vitamins (vitamins C and E and provitamin A) and selenium, and solubilized ubiquinone Q_{10} on the antioxidant potential in rat liver (ascorbate-dependent free radical oxidation of unsaturated membrane phospholipids). β-Carotene irrespective of the administration route increased antioxidant potential of the liver by 2-3.5 times. The complex of antioxidant vitamins and selenium increased this parameter by more than 15 times. Antiradical activity in rat liver was extremely high after administration of solubilized ubiquinone Q_{10} (increase by more than by 36 times). It can be expected that reduced ubiquinone Q_{10} *in vivo* should produce a more pronounced protective effect due to activity of the system for bioregeneration of this natural antioxidant.

Key Words: *free radical lipid oxidation; ubiquinone Q_{10}; antioxidant vitamins; tissue antioxidant potential*

Oxidative stress accompanied by free radical oxidation of unsaturated lipids in biological membranes produces changes in their conformation and activity of membrane-bound enzymes [5,9,11,12]. Homolysis of lipid hydroperoxides leads to accumulation of carbonyl compounds (mainly aldehydes) damaging or modifying molecules of biopolymers, including proteins and nucleic acids [5,9,11,12]. These changes play an important role in the etiology and pathogenesis of various diseases [2,5,6,13]. The search for potent antioxidants protecting biological membranes of living cells from oxidative stress-produced damage is an urgent problem [1,2,6]. Natural antioxidants holds much promise for pharmacological treatment due to the absence of toxic activity and bioregeneration in the organism [1,2,5]. Here we studied the effects of peroral treatment with natural antioxidants β-carotene, ubiquinone Q_{10}, and complex of antioxidant vitamins (vitamins C and E and provitamin A) and selenium on the antioxidant potential in rat liver (period of ascorbate-dependent oxidation in biological membranes).

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MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 260±10 g. The animals were divided into 4 groups. Aqueous suspensions or oil and aqueous solutions of antioxidant preparations (total volume 0.5 ml) were administered daily for 30 days through an tube. Group 1 rats (n=8) received fine suspension of β-carotene in distilled water (20 mg/kg, Sigma). Group 2 rats (n=7) received the same preparation in sunflower oil. Group 3 rats (n=9) received fine aqueous suspension of antioxidant vitamins and selenium (Triovit, KRKA). It was prepared from the content of a capsule with a commercial preparation (40 mg/kg α-tocopheryl ace-

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tate, 100 mg/kg ascorbic acid, 10 mg/kg β-carotene, and 50 μg/kg organic compounds of yeast selenium). Group 4 rats received water-solubilized ubiquinone Q_{10} in a dose of 10 mg/kg (Kudesan, Akvion). The animals of 4 control groups (*n*=8, 26, 10, and 8, respectively) received an equivalent volume of solvents. The effective dose of antioxidants was determined from previously estimated concentration dependence for antioxidant activity of β-carotene in rat liver [8]. By the end of the experiments the rats were narcotized and decapitated. The liver was perfused with cold isotonic KCl and homogenized in an Ultra-Turrax SDT-1810 tissue microhomogenizer (Tekmar) under cooling (15 mg wet tissue per 1 ml solution containing 0.154 M NaCl and 50 mM K/Na phosphate buffer, pH 5.9). The homogenates were incubated with 0.5 mM ascorbate under aerobic conditions and constant shaking (without adding Fe^{2+} supply) [4]. Aliquots of the incubation mixture were taken at fixed time intervals (1-5 min). The content of secondary lipid peroxidation products was estimated in the reaction with thiobarbituric acid (TBA). Optical density of samples was measured on a Hitachi 557 spectrophotometer at 532 nm [4]. The initial absorption of TBA-reactive substances estimated before incubation was subtracted from optical density of samples. The lag phase of oxidation (induction period, τ) was calculated from kinetic curves constructed by ΔD_{532} [4]. The induction period in various groups of control animals (τ_0) corresponded to 89.0± 20.6 sec. We revealed no significant intergroup differences in τ_0. For correct comparison the data for experimental rats were expressed relative to the control value (τ/τ_0).

RESULTS

We previously showed that the method for studying ascorbate-dependent oxidation of unsaturated membrane phospholipids adequately characterizes the intensity of free radical processes *in vitro* [4]. The test antioxidants increased the lag phase of ascorbate-dependent oxidation of unsaturated phospholipids in biological membranes of rat liver (Table 1). Similarly to commercial preparations of antioxidant vitamins, the complex antioxidant preparation includes esterified α-tocopherol (α-tocopheryl acetate, group 3 animals). As differentiated from free α-tocopherol (α-TOH), α-tocopheryl acetate (α-TOR) has no antioxidant activity. Interaction with the free radical of lipid hydroperoxide ($LO_2^{\bullet}$) should be accompanied by detachment of the hydrogen atom from a free OH group in the carbon atom of the chroman nucleus in the phenol molecule. However, it does not occur after esterification:

$$LO_2^{\bullet}+\alpha\text{-TOH}\rightarrow LOOH+\alpha\text{-TO}^{\bullet} \quad \textbf{(1)},$$
$$LO_2^{\bullet}+\alpha\text{-TOR}\rightarrow(\text{reaction is impossible})$$

In our study potential antioxidant α-tocopheryl acetate could *in vivo* exhibit antiradical activity only after hydrolysis of the ester bond with pancreatic or hepatic carboxy ester hydrolases [6]. Consumption of selenium by group 3 animals probably contributed to expression of the antioxidant enzyme glutathione peroxidase [5,6,10]. High antioxidant activity (15-fold increase relative to the control) of the complex preparation in free radical oxidation of biological membranes from the liver of group 3 rats is probably related to the presence of these pro-antioxidant components. Another membranotropic antioxidant β-carotene in a 2-fold higher dose produced less pronounced antiradical effect in group 1 and 2 animals (increase in the τ/τ_0 ratio by 2-3.5 times irrespective of the administration route, Table 1). Administration of ubiquinone Q_{10} significantly increased the antioxidant potential of biological membranes (by more than 36 times, Table 1). Probably, the quinone form of coenzyme Q_{10} (Q) did not exhibit antioxidant activity in group 4 animals. Ubiphenols formed during reduction in the mitochondrial electron transport chain or during interaction with ascorbate ($^{\bullet}OH$, QH_2) probably gain antioxidant properties [1,2,5,6]. As differentiated from α-tocopherol, biphenol (QH_2) formed after two-electron reduction of ubiquinone Q_{10} can neutralize 2 lipid free radicals. This reaction is accompanied by the formation of a ubisemiquinone radical as an intermediate product ($^{\bullet}QH$):

TABLE 1. Antioxidant Potential in Rat Liver after Administration of Preparations Containing Major Natural Antioxidants (*M*±*m*)

Group	Antioxidants	Relative induction periods, τ/τ_0
1	β-Carotene (finely dispersed aqueous suspension)	3.40±0.41 (8)
2	β-Carotene (solution in sunflower seed oil)	2.10±0.35 (7)
3	Complex of antioxidant vitamins and selenium (finely dispersed aqueous suspension)	15.10±0.25 (9)
4	Ubiquinone Q_{10} (water-solubilized form)	36.10±0.33* (10)

Note. Number of animals is shown in brackets. *$p<0.05$ compared to group 3.

$$LO_2^{\bullet}+QH \rightarrow LOOH+{}^{\bullet}OH \quad \textbf{(2)},$$
$$LO_2^{\bullet}+{}^{\bullet}QH \rightarrow LOOH+Q \quad \textbf{(3)}.$$

A similar mechanism mediates ubiphenol-dependent bioregeneration of phenoxyl free radicals of α-tocopherol (α-TO$^{\bullet}$) formed during the interaction of vitamin E (α-TOH) with lipid radicals **(1)**:

$$\alpha\text{-}TO^{\bullet}+QH \rightarrow \alpha\text{-}TOH+{}^{\bullet}QH \quad \textbf{(4)},$$
$$\alpha\text{-}TO^{\bullet}+{}^{\bullet}QH \rightarrow \alpha\text{-}TOH+{}^{\bullet}Q \quad \textbf{(5)}.$$

It can result in reduction of 2 tocopheroxyl radicals (**2** and **3**; **4** and **5**). Extremely high antioxidant activity of ubiquinone Q_{10} is mainly related to these antiradical properties and possible α-tocopherol-preserving effect (Table 1). It can be hypothesized that reduced ubiquinone Q_{10} *in vivo* should produce a more pronounced protective antiradical effect due to activity of the system for bioregeneration of this natural antioxidant. It should be emphasized that ubiphenol Q_{10}, but not α-tocopherol, serves as the major antioxidant determining oxidation resistance of atherogenic low-density lipoproteins [5,6,15]. In our experiments the highest antiradical activity of ubiquinone Q_{10} was probably related to its complete reduction in the incubation medium with 0.5 mM ascorbate (HO—Asc—OH). This reaction proceeds with the formation of an intermediate product semidehydroascorbate (HO—Asc—O$^{\bullet}$):

$$^{\bullet}QH+HO—Asc—OH \rightarrow HO—Asc—O^{\bullet}+QH_2 \quad \textbf{(6)},$$
$$^{\bullet}QH+HO—Asc—O^{\bullet} \rightarrow O=Asc=O+Q \quad \textbf{(7)}.$$

Our results indicate that ubiquinone Q_{10} produced most pronounced antioxidant effect in animals compared to preparations containing other natural fat-soluble antioxidants (Table 1). Ubiquinone Q_{10} holds much promise to inhibit oxidative stress *in vivo*. This conclusion is supported by the results of our previous studies. We showed that ubiquinone Q_{10} *in vivo* diminishes the prooxidant effect of cholesterol-reducing drugs belonging to the statin family [7,14,15]. Moreover, pretreatment with ubiquinone Q_{10} protects the myocardium from oxidative stress [3].

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