PHARMACOLOGY AND TOXICOLOGY

β-Carotene-Containing Preparations Enhance Antioxidant Potential of the Liver and Myocardium

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The effects of β-carotene-containing food additives carinate and carinate CD on the antioxidant potential of rat liver and myocardium were examined. Daily oral administration of these drugs in doses equal to 0.4 and 14 mg/kg β-carotene inhibited ascorbate-dependent peroxidation of endogenous lipids in hepatocytes and cardiomyocytes 1.5-6.5- and 1.5-40-fold, respectively, depending on β-carotene form and dose. Carinate CD containing a complex of β—carotene with β-cyclodextrin was a more potent inhibitor of lipid peroxidation in the liver and myocardium than carinate containing free β-carotene. β-Carotene-containing food additives can be recommended for the prophylaxis of cardiovascular, oncological, and other diseases.

Key Words: *β-carotene; antioxidants; prooxidants; lipids; free radical oxidation*

Provitamin A or β-carotene is a component of many food additives and multivitamin preparations. β-Carotene is assigned to the group of natural antioxidants because of its ability to inhibit free radical oxidation of different substrates in model systems [6-9]. However, *in vivo* antioxidant effect of β-carotene has not been studied in detail. In the present study we examined the changes in duration of lag-phase of free radical ascorbate-dependent oxidation of endogenous lipids in rat liver and myocardium after 30-day oral administration of β-carotene and carotene-containing additives containing provitamin A in a nonimmobilized form or as a complex with β-cyclodextrin.

MATERIALS AND METHODS

Experiments were carried out on 106 male Wistar rats weighing 180±20 g. The rats received 0.5 or 20 mg/kg β-carotene or β-carotene-containing food additives (carinate and carinate CD) in doses equal to 0.4 and 14 mg/kg β-carotene during 1 month. β-Carotene (0.2 ml in olive oil) and β-carotene-containing additives (tablets suspended in 1 ml water) were administered through a tube. Each tablet of complex carinate preparation (Institute of Atherosclerosis, Moscow) contains 150 mg garlic powder, 2.5 mg β-carotene, 1 mg vitamin E (α-tocopherol acetate), and 1 mg vitamin C (ascorbic acid). Carinate CD contains the same ingredients but β-caroten is replaced by cyclocar (Vitamins, Moscow) [4], presenting a complex of β-carotene with β-cyclodextrin.

Control animals received equal volumes of olive oil or distilled water according to the same scheme.

At the end of the experiments the rats were decapitated, the liver was perfused, and the myocardium was washed thoroughly with cold KCl isotonic solution. The liver and myocardium were homogenized in cold 50 mM Na,K-phosphate buffer (pH 5.9) containing 0.154 M NaCl at a ratio of 15 mg raw tissue/ml buffer in an "Ultra-Turrax SDT-1810" tissue homogenizer (Tekmar). Tissue homogenates were incubated on a shaker under aerobic conditions in the presence

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TABLE 1. Effects of 1-Month Oral Administration of β-Carotene and β-Carotene-Containing Drugs on lag-Phase of Free Radical Ascorbate-Dependent Oxidation of Endogenous Polyenic Lipids in Rat Liver and Myocardium Homogenates

Preparation, dose (mg/kg)	lag-phase, sec (% of control)	
	liver	myocardium
Control (*n*=26)	104±10.8 (100)	294±33.1 (100)
β-Carotene, 0.5 (*n*=7)	177±20.1* (172)	753±71.3* (256)
Carinate, 0.4 (*n*=17)	143±14.5* (139)	451±42.0* (153)
Carinate CD, 0.4 (*n*=15)	220±14.9*+ (214)	989±52.5*+ (336)
β-Carotene, 20 (*n*=7)	159±23.7* (154)	790±125.5* (269)
Carinate, 14 (*n*=18)	174±20.2* (169)	>10 800** (>3670)
Carinate CD, 14 (*n*=16)	666±72.4*+ (647)	>10 800** (>3670)

Note. $p<0.05$ compared to control (*) or carinate (+). **Exact duration of lag-phase was not determined because oxidation was not stimulated during 3-h incubation.

of 0.5 mM ascorbate [1]. Aliquotes taken after 1-5-min incubation were assayed for the presence of secondary lipid peroxidation (LPO) products by the reaction with thiobarbituric acid (TBA). Optical density was measured on a Hitachi 557 spectrophotometer at 532 nm [1,2]. Initial absorbtion was extracted from optical density of subsequent samples and kinetic curves and the duration of lag-phase were calculated on the base of ΔD_{532} [1,2].

RESULTS

Daily administration of nonimmobilized β-carotene or test β-carotene-containing drugs during 30 days significantly increased antioxidant potential of rat myocardium and liver (Table 1). Initial content of natural antioxidants in rat myocardium (as determined by oxidation lag-phase) 3-fold surpassed that in the liver. Antioxidant effect of nonimmobilized β-carotene and β-carotene-containing drugs was more pronounced in the myocardium than in the liver (Table 1). The most pronounced antioxidant effect on hepatocytes and cardiomyocytes was achieved with carinate CD in doses equal to 0.4-0.5 mg/kg β-carotene; no significant differences in the effects of β-carotene oil solution and carinate were found. In higher doses (14-20 mg/kg) β-carotene and carinate produced similar effects on hepatocytes, whereas the antioxidant effect of carinate CD in this tissue was significantly (6.5-fold) higher. β-Carotene (20 mg/kg) administered daily during 1 month enhanced antioxidant potential of cardiomyocytes 2.5-fold, while carinate and carinate CD in doses equal to 14 mg/kg provitamin A almost completely inhibited free radical oxidation in the myocardium (lag-phase of ascorbate-dependent LPO exceeded 3 h *vs.* 5 min in the control: in some experiments we observed no accumulation of malonic dialdehyde after 10-h oxidation). Despite the presence of natural garlic antioxidants [3] and antioxidant vitamins E and C, the *in vivo* effect of carinate and carinate CD primarily depends on β-carotene. Thus, the antioxidant potential of hepatic tissue did not depend on the drug form of β-carotene (apart from carinate CD in a dose equivalent to 14 mg/kg β-carotene), which points to insignificant antioxidant activity of other carinate and carinate CD components. Since carinate CD differs from carinate only by complexation of β-carotene with β-cyclodextrin that possesses no antioxidant properties, the significant differences between these drugs suggest that their antioxidant activity is determined mainly by β-carotene. Thus, experiments demonstrated a pronounced antioxidant effect of optimal concentrations of β-carotene *in vivo*, especially in the myocardium [2]. Moreover, complexation of β-carotene with β-cyclodextrin significantly enhances its antioxidant properties. Our results indicate high bioavailability of β-carotene in a complex with β-cyclodextrin, which is of special importance in view of low absorption of β-carotene from carotene-rich vegetables [5] and prophylactic significance of β-carotene-containing drugs. Thus, optimal doses of β-carotene-containing food additives and multivitamin preparations with pronounced antioxidant effect can be recommended for complex therapy of cardiovascular, oncological, and other diseases associated with oxidative stress.

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