Concentration-Dependent Inversion of Antioxidant and Prooxidant Effects of β-Carotene in Tissues *In Vivo*

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Low doses (below 20 mg/kg) of oral β-carotene inhibit, while high doses (above 100 mg/kg) activate ascorbate-dependent LPO in rat liver and myocardial cells. Administration of β-carotene in a dose of 20 mg/kg decreased by 34% infarction zone in coronary occlusion, while the dose of 100 mg/kg was ineffective. An *in vivo* concentration-dependent inversion of β-carotene from antioxidant to prooxidant in tissues is hypothesized. Pharmacological efficacy of β-carotene is determined by its antioxidant effect, while high doses provoke the prooxidant effect and can lead to negative consequences.

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

Provitamin A (β-carotene) is an essential nutrient. This isoprenoid compound contains a system of four conjugated dienes, and being a polyenic lipid is readily oxidized by the free-radical mechanism [5,7]. Thus, β-carotene acts as a prooxidant and provokes free-radical oxidation of other polyunsaturated lipids in biomembranes. However, β-carotene can directly interact with free radicals [5,8] and inhibit radical oxidation of various substrates [5,9]. The mechanism of antioxidant effect of β-carotene differs from that of classical natural phenol antioxidants (α-tocopherol) and apparently consists in the production of inactive carbon-centered resonance radicals in the isoprenoid chain upon interaction with active hydroperoxide radicals [5]. β-Carotene can be referred to natural antioxidants and used for the treatment of cardiovascular diseases and malignant growth [5, 11,14]. Reports about clinical use of β-carotene are contradictory [5,10,11,13,14]. We assumed that opposite *in vivo* effects are due to different doses used: β-carotene in different concentrations can act as a antioxidant and a prooxidant.

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MATERIALS AND METHODS

Male Wistar rats weighing 180±20 g were used. Group 1 animals (n=44) daily received β-carotene in olive oil (200 μl/rat) through a tube in doses of 0.5, 20, 50, or 100 mg/kg (for administration of 50 and 100 mg/kg a fine suspension of β-carotene was used). In group 2 (n=111) myocardial infarction (MI) was modeled by occlusion of the left coronary artery under endotracheal ether narcosis [2]; ischemia and MI in operated rats were confirmed by ECG. β-Carotene in olive oil in doses of 20 or 100 mg/kg was administered orally 1 day before surgery, 2 h before occlusion, and daily for 7 days postoperation. Control animals for both groups were administered the same volume of olive oil according to the same protocol. After experiment, group 1 animals were decapitated, the liver was perfused, and the heart was thoroughly washed in cold isotonic KCl. The liver and myocardium were homogenized with cooling in an Ultra-Turrax SDT-1810 microgrinder (Tekmar) (15 mg wet tissue/ml solution containing 0.154 M NaCl and 50 mM K,Na-phosphate buffer, pH 5.9). The homogenates were incubated at constant shaking under aerobic conditions with 0.5 mM ascorbate [3]. In aliquots taken every 1-5 min, the

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concentrations of LPO products were assayed by reaction with thiobarbituric acid (TBA), optical density was measured at 532 nm in a Hitachi-557 spectrophotometer [3]. The initial absorption of TBA-reactive products (before incubation) was subtracted from optic density of subsequent samples, and ΔD_{532} kinetic curves were plotted, from which the duration of the lag phase was calculated [3]. Animals with MI were sacrificed on day 7 postoperation; for evaluating the size of cicatricial zone, succinate dehydrogenase activity was measured on frozen heart slices by NBT reduction (involved myocardial zone remained unstained). The percentage of tissue lesion was evaluated planimetrically by IBAS-1 (Opton), and the total weight of cicatricial tissue was calculated from heart weight and percentage of involvement [2].

RESULTS

β-Carotene in doses of 0.5 and 20 mg/kg markedly inhibited LPO in the liver and myocardium, which is reflected by a 2-2.5-fold prolongation of the lag-phase (Fig. 1). Hence, the maximum antioxidant effect of β-carotene in the liver and myocardium *in vivo* can be caused by administration of 0.5-20 mg/kg. On the other hand, in doses of 50 and 100 mg/kg β-carotene notably activated LPO in the liver and myocardium and shortened the lag-phase. This prooxidant effect of β-carotene was most pronounced in the liver: in the majority of cases oxidation was not preceded by the induction period (Fig. 1). These data agree with the effect of β-carotene on the size of MI zone in experimental rats. Oral β-carotene in a dose of 20 mg/kg exerted an antinecrosogenic effect in rats with coronary occlusion (MI zone decreased by 34%), while a higher dose (100 mg/kg) was ineffective (Table 1). This correlation between biochemical and pharmacological findings is not accidental, because β-carotene had an antinecrosogenic effect in MI only in a dose inducing a sharp increase of the antioxidant potential in the myocardium (Fig. 1, Table 1). And no pharmacological activity of β-carotene was observed in doses provoking its prooxidant effect (Fig. 1, Table 1). Our findings explain the controversy on the therapeutic effect of β-carotene in cardiovascular patients [10, 11,13]. Prooxidant effect of β-carotene may be explained by the fact that it is a good substrate for free-radical oxidation and, if present in sufficient concentrations, may become a potent source of free radical initiating LPO [5,7]. Therefore, the results of biochemical and pharmacological experiments indicate the possibility of a dose-dependent inversion of antioxidant into prooxidant effect of β-carotene *in vivo*. The concentration-dependent inversion may be characteristic of other antioxidant vitamins as well. The main

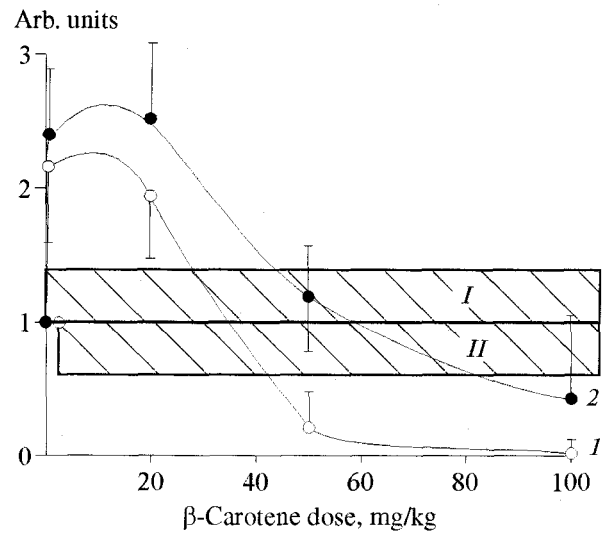

Fig. 1. Antioxidant (*I*) and prooxidant (*II*) effects of β-carotene *in vivo* on oxidation of endogenous polyene lipid of the liver (*1*) and myocardium (*2*) in rats. Cross-hatched areas: 95% confidence intervals for control animals. Ordinate: oxidation lag phase, arb units.

natural antioxidant α-tocopherol can provoke a prooxidant effect due to accumulation of LPO-initiating α-tocopheroxyl radicals [4]. Ascorbate also can realize antioxidant or prooxidant functions in biological systems due to reduction of α-tocopherol radicals or Fe^{3+} [1,12]. Even a synthetic "alimentary" phenol antioxidant butyrilated hydroxyanisole can exert a prooxidant effect due to generation of superoxide radicals during reaction of its metabolites with molecular oxygen [6]. It should be noted that for all substances mentioned, including β-carotene, inversion of the antioxidant into prooxidant effect is observed upon increasing their concentrations. These findings suggest the existence of threshold concentrations causing inversion of the antioxidant function; this can impede prediction of the pharmacological effect of antioxidant vitamins and synthetic antioxidants in patients. Fatal danger of surpassing the optimal physiological concentrations of β-carotene in tissues is obvious. However, the documented relationship between carotenoid deficiency in the organism and risk of malignant tumors and cardio-

Table 1. Effect of Oral Administration of β-Carotene in Various Doses on the Size of Cicatricial Zone of MI in Rats on Day 7 after Occlusion of the Left Coronary Artery

β-Carotene dose, mg/kg	Size of MI zone, %	
	of heart weight	of control
0 (*n*=80)	23.0±0.83	100±3.6
20 (*n*=21)	15.1±1.60*	66±7.0*
100 (*n*=10)	21.4±0.84	93±3.6

Note. *$p<0.05$ *vs.* control.

vascular disease [10,11,13,14] dictate regular prophylactic intake of low doses of β-carotene (approximating the scientifically-based daily requirement in provitamin A in certain regions) for replenishing its deficit in the organism.

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