Antioxidant Activity of Parapharmaceutics Containing Natural Inhibitors of Free Radical Processes

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The duration of lag phase of ascorbate-dependent free radical oxidation of endogenous polyenic lipids in rat liver and myocardium considerably increased after oral administration of lacrinat containing licorice *Glycyrrhiza glabra* root powder for 1 month. Lacrinat markedly decreased the content of lipid peroxides in rat liver. *Ex vivo* antioxidant effects of lacrinat in rat liver were comparable with those of β-carotene-containing preparations carinat and carinat CD. Parapharmaceutics containing both licorice *Glycyrrhiza glabra* root powder and β-carotene (carinat forte) markedly increased antioxidant activity of the liver.

Key Words: *antioxidants; free radical lipid peroxidation; β-carotene; glycyrrhizine; flavonoids; licorice Glycyrrhiza glabra root*

Antioxidants are now proposed as radio- and geroprotectors for the complex therapy of cardiovascular disorders [2]. Parapharmaceutics containing natural antioxidants, inhibitors of free radical processes, hold much promise in this respect [2]. Licorice *Glycyrrhiza glabra* (LR) root contains various isoflavonoids, including glabridine and its derivatives, possessing high antioxidant activity [5,8,9]. Natural antioxidant β-carotene (provitamin A) is an essential component of all multivitamin preparations. However, isoprenoid chain of β-carotene is easily oxidized under aerobic conditions and, therefore, this unsaturated compound has to be stabilized [3,6]. Complexes of β-carotene and β-cyclodextrin [7] improve not only the stability of immobilized β-carotene, but also its biological availability [6]. Oxidative degradation of β-carotene in medicinal preparations can also be prevented by natural or synthetic antioxidants. It should be emphasized that β-carotene displays antioxidant activity *in vivo*, but in high concentrations this substance exhibits prooxidant properties [3]. The use of natural antioxidants (e.g., vitamin E) for stabilization of β-carotene is not always appropriate, because phenoxy radicals formed during oxidation of α-tocopherol promote free radical oxidation of β-carotene [2,11]. At the same time, it is believed that natural LR antioxidants prevent oxidative degradation of β-carotene in the composition of drugs *in vitro* and in cells *in vivo*. Here we studied free radical lipid oxidation in the liver and myocardium of rats orally treated with preparations containing β-carotene and LR powder for 1 month.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180±20 g and divided into 4 groups of 6-8 animals each. The animals daily received through a gastric tube (*per os*) 0.5 ml water suspensions of nutriceutics carinat and carinat CD containing β-carotene (2 mg β-carotene/100 g body weight), lacrinat containing LR powder (200 mg/100 g body weight), or carinat forte containing β-carotene and LR powder for 30 days (Inat-Farma, Table 1). Control rats (n=8) received an equivalent volume of water. The animals were decapitated under anesthesia, the liver was perfused, and the heart was thoroughly washed with isotonic KCl. The liver and myocardium (15 mg wet tissue/ml) were cooled and homogenized in a medium contain-

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ing 0.154 M NaCl and 50 mM K,Na-phosphate buffer (pH 5.9) using an Ultra-Turrax SDT-1810 homogenizer (Tekmar). Tissue homogenates were incubated with 0.5 mM ascorbate under constant shaking and aerobic conditions [1,3]. The incubation medium was sampled at 1-5-min intervals, and the content of secondary products of lipid peroxidation was estimated by the reaction with thiobarbituric acid (TBA). Optical density of samples was measured on a Hitachi 557 spectrophotometer at 532 nm [1]. The initial absorption of TBA-reactive products (before incubation) was subtracted from the optical density of samples measured after incubation (ΔD_{532}), and the kinetic curve for evaluating the lag phase was plotted [1]. Aliquots of liver homogenates (0.5 ml) were extracted with the chloroform-methanol mixture (volume ratio 2:1) by the method of Folch. Chloroform was evaporated in vacuum and then in an argon flow to constant weight of lipid extracts. The content of lipids was measured gravimetrically. Lipids were dissolved in methanol, and the content of lipid hydroperoxides was estimated by the reaction of Fe^{3+} with xylenol orange before and after reduction of organic hydroperoxides with triphenylphosphine on a Hitachi 557 spectrophotometer at 560 nm [12].

RESULTS

Lacrinat administered for 30 days markedly inhibited ascorbate-dependent oxidation of endogenous polyenic lipids (ADOEL) in rat liver and myocardium (Fig. 1, *a*, *b*). The lag phase of ADOEL in rat liver after treatment with lacrinat increased from 321±77 to 878±209 sec ($p<0.05$). This effect of lacrinat is similar to that of β-carotene-containing carinat (lag phase 960±161 sec, $p<0.05$, data not shown). The duration of ADOEL in the myocardium of lacrinat-treated rats considerably increased so that even after 3-h incubation we found no oxidation products in the medium (Fig. 1, *b*). Carinat produced similar changes (data not shown), which is consistent with published data [6]. *In vitro* experiments indicate that LR contains inhibitors of free radical processes strengthening the antioxidant capacity of tissues. The data suggest that *in vivo* treatment with LR powder markedly inhibits free radical processes. Lacrinat decreased the content of lipid peroxides in rat liver by 25% ($p<0.05$, Table 2). Furthermore, carinat forte containing β-carotene and LR powder displayed more potent antioxidant effects: the content of lipid peroxides in the liver decreased by 2 times compared to the control. Our previous experiments showed that carinat and, in particular, carinat CD considerably increased the lag phase of ADOEL in rat liver and myocardium [6]. At the same time, carinat and carinat CD were equally potent in decreas-

TABLE 1. Composition of Test Parapharmaceutics

Preparation	Composition (in 1 tablet)
Carinat	2.5 mg β-carotene, 5 mg vitamin E, 30 mg vitamin C, 150 mg garlic powder
Carinat CD	The same as in carinat except for β-carotene is presented by cyclokar (complex of β-carotene and β-cyclodextrin) [6,7]
Lacrinat	200 mg LR powder
Carinat forte	The same as in carinat+200 mg LR powder

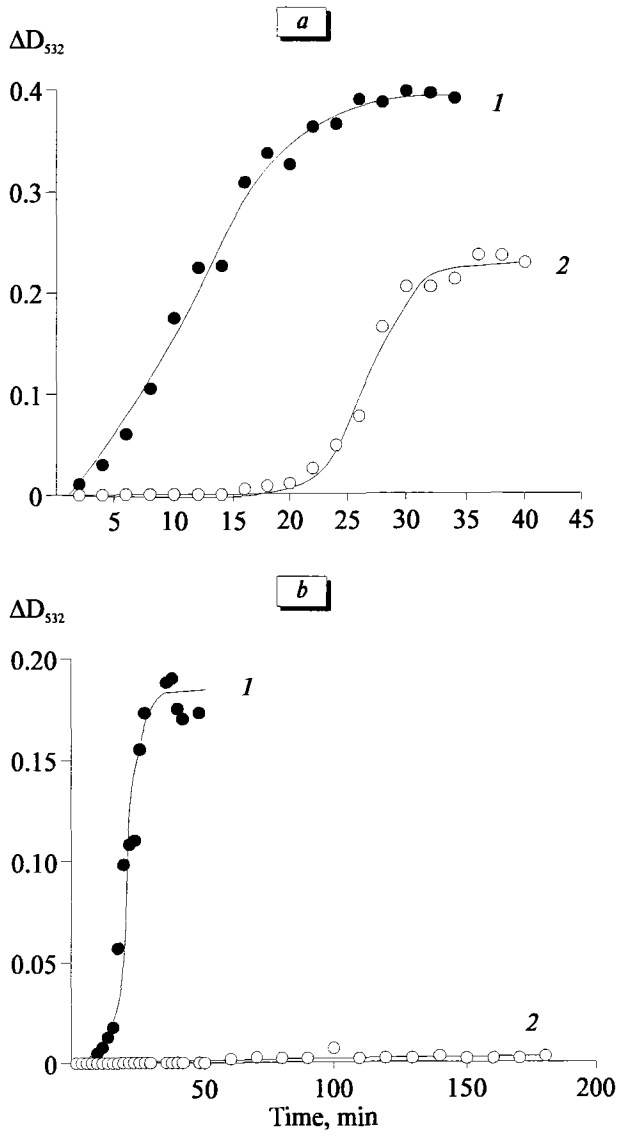

Fig. 1. Kinetics of ascorbate-dependent free radical oxidation of endogenous polyenic lipid in the liver (*a*) and myocardium (*b*) of control rats (*1*) and in the liver of rats orally treated with licorice *Glycyrrhiza glabra* root powder for 1 month (*2*).

TABLE 2. Content of Lipid Hydroperoxides (nmol/g total lipids) in the Liver of Rats Orally Treated with Nutrients Containing LR Powder and β-Carotene for 1 Month ($M \pm m$)

Control	Carinat (n=6)	Carinat CD (n=7)	Lacrinat (n=7)	Carinat+lacrinat (n=7)
9.70±0.56	6.30±1.37*	6.70±0.42*	7.40±0.69*	4.4±0.4*

Note. *p<0.05 compared to the control.

ing the content of lipid peroxides in rat liver (Table 2). It was shown that isoflavonoids of LR (glabridine and its derivatives) inhibit free radical oxidation of low-density lipoproteins in human plasma *in vitro* [8, 9,11]. Other isoflavonoids entering the composition of LR (licochalcones B and D) inhibit NADPH-dependent oxidation of microsomal membranes, prevent $O_2^{\bullet}$ formation during oxidation of xanthine with xanthine oxidase, and protect erythrocytes from oxidative hemolysis [10]. LR extracts also inhibit free radical processes induced by ionizing radiation [4]. Hence, our findings are consistent with published data and confirm that LR contains potent natural antioxidants. Therefore, LR powder can be used for prevention of provitamin A in β-carotene-containing nutriceutics from oxidation. Moreover, these preparations *in vivo* enhance antioxidant effects of β-carotene.

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