Dose-Dependent Effect of α-Tocopherol on Activity of Xenobiotic Metabolizing Enzymes in Rat Liver

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Oral treatment with α-tocopherol for 4 days dose-dependently increased the content of cytochrome P450 (CYP), catalytic activities of CYP1A1, CYP1A2, CYP2B1, CYP2C, and activity of NADPH-cytochrome-P450 reductase in the liver of male rats, but did not change activity of glutathione S-transferase. These results suggest that α-tocopherol induced the enzymes of phase I of xenobiotic metabolism, including CYP1 and CYP2 families involved in the metabolism of drugs and procarcinogenes.

Key Words: *α-tocopherol; CYP1A; CYP2B; CYP2C; NADPH-cytochrome-P450 reductase; glutathione S-transferase*

α-Tocopherol (α-TP), the most active form of vitamin E, is widely used as an antioxidant for the prevention of diseases caused by oxidative stress (inflammatory diseases, atherosclerosis, and cancer). Recent studies revealed a number of functions of α-TR in cell metabolism, which are not associated with its well-studied antioxidant and antiradical activities and cannot be performed by other tocopherols. Among these functions are induction of phospholipase A2 and cyclooxygenase, inhibition of proteinkinase C and 5-lipoxygenase at the posttranslation level, modulation of expression of CD36, α-TP-transporting protein, and α-tropomyosin genes [12].

Cytochrome P450 (CYP), NADPH-cytochrome-P450-reductase, and glutathione S-transferase are enzymes of phases I and II of metabolism of hydrophobic endogenous compounds and xenobiotics mediating both detoxication and toxification reactions [13]. Induction of these enzymes by various xenobiotics is well studied, but little is known on the effects of vitamins on these enzymes. It is known that α-TP-deficient diet leads to a considerable decrease in the total content of cytochrome P450 in rat liver [6], while addition of α-TP to the ration increases activities of benz(a)pyrene-hydroxylase [14], CYP2C11 [9], CYP2B1 and GST [2] in rat liver microsomes.

We studied the effects of various oral doses of α-TP on phase I and phase II xenobiotic metabolism enzymes, including the effects on activities of CYP2B and CYP2C families metabolizing various drugs drugs, and of CYP1A family involved in activation of many procarcinogenes.

MATERIALS AND METHODS

The study was carried out on 30 male Wistar rats (100-120 g) kept under standard laboratory diets and starving for 24 h before sacrifice. Experimental animals received α-TP acetate orally in doses of 10, 30, 70, 150, and 300 mg/kg (in 30% oil) for 4 days and were decapitated on day 5. The cytosol fraction and liver microsomes were isolated at 4°C by differential centrifugation. The content of protein in microsomes and cytosol was measured by the method of Lowry with BSA as the reference protein. The total content of cytochrome P450 was measured by the method proposed by Omura and Sato [10]. The rate of O-dealkylation of substrates (7-pentoxy-, 7-ethoxy-, 7-methoxy, and 7-benzoxyresorufins) highly specific for rat

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CYP2B1, CYP1A1, CYP1A2, and CYP2C, respectively, were measured in liver microsomes by the fluorimetric method by the rate of resorufin generation [1]. Activity of NADPH-cytochrome-P450-reductase in liver microsomes was evaluated by the rate of cytochrome *c* reduction [11]. Tocopherol content in rat liver microsomes was measured by the fluorimetric method after their extraction with hexane [4]. Calibration curves were plotted using ethanol solutions of α-TP as the standards. Activity of glutathione S-transferase in the cytosol was measured by the rate of S-(2,4-dinitrophenyl)glutathione production with 1-chloro-2,4-dinitrobenzene as glutathione acceptor and S-(2,4-dinitrophenyl)glutathione as the reference agent [3].

The data were statistically processed using Statistica software (version 5.5). The significance of differences was evaluated using Student's *t* test and confirmed by the Mann—Whitney *U* test.

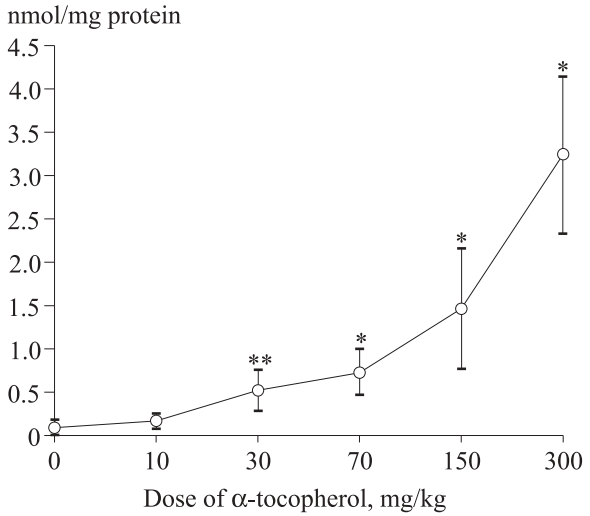

Fig. 1. Changes in the content of total tocopherols in liver microsomes of rats treated with α-tocopherol. *p<0.001, **p<0.01 compared to the control.

RESULTS

The total content of tocopherols in rat liver microsomes increased with increasing the administered dose of α-TP (Fig. 1) and attained a 36-fold increase at a dose of 300 mg/kg. Tocopherol content increased 1.87 fold in comparison with the control after treatment with a dose of 10 mg/kg, though significant differences in this case were detected only by Mann—Whitney test (p<0.027).

The total content of cytochrome P450 in liver microsomes of rats receiving α-TP in doses of 10, 70, and 300 mg/kg 1.7-, 2.2-, and 2.1-fold surpassed the control, respectively, but did not differ from the control in rats receiving 30 and 150 mg/kg α-TP (Fig. 2, *a*). Activity of NADPH-cytochrome-P450 reductase in microsomes increased 2-fold in comparison with the control only after administration of 300 mg/kg α-TP (Fig. 2, *b*). α-TP in all doses had no effect on glutathione S-transferase activity (Fig. 2, *c*). However, we observed a tendency to its increase at α-TP doses of 10 and 30 mg/kg.

7-Pentoxyresorufin-O-dealkylase activity characterizing CYP2B1 (Fig. 3, *a*) increased 2.7, 4.8, and 9.9 times after treatment with α-TP in doses of 30, 70, and 300 mg/kg, respectively, in comparison with the control, and virtually did not change after treatment in doses of 10 and 150 mg/kg. 7-Benzylresorufin-O-dealkylase activity characterizing the total activity of CYP2B and CYP2C (Fig. 3, *b*) increased 4.5, 8.2, 11.4, 4.5, and 6.9 times after treatment with α-TP in doses of 10, 30, 70, 150, and 300 mg/kg, respectively, in comparison with the control. 7-Ethoxyresorufin-O-dealkylase activity, a marker for CYP1A1 (Fig. 3, *c*), increased 2.6, 5.4, 2.0, and 2.5 times after treatment with

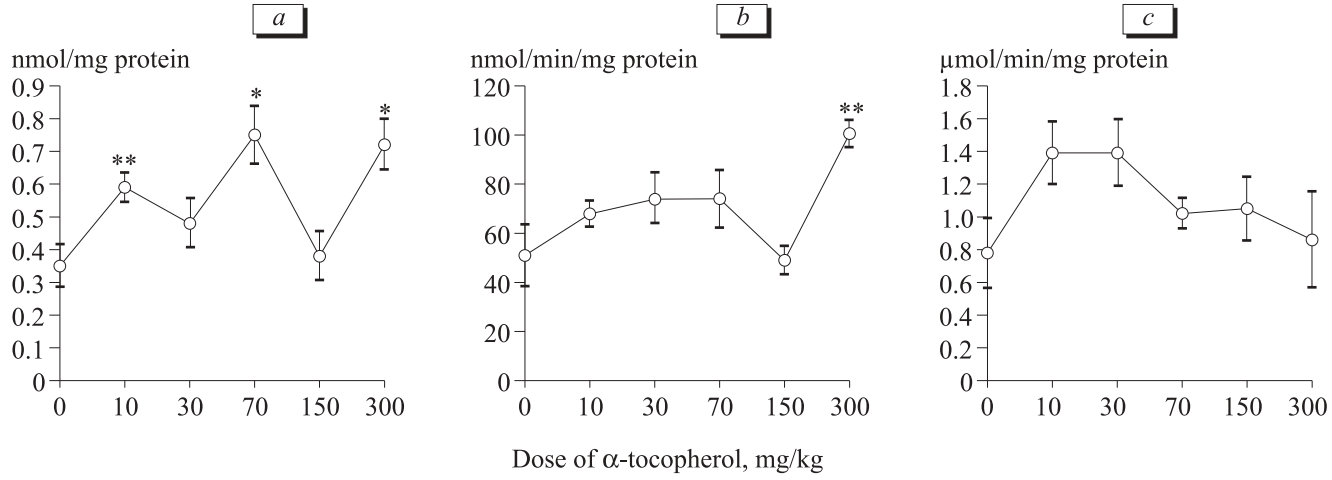

Fig. 1. Changes in the content of total tocopherols in liver microsomes of rats treated with α-tocopherol. *p<0.001, **p<0.01 compared to the control.

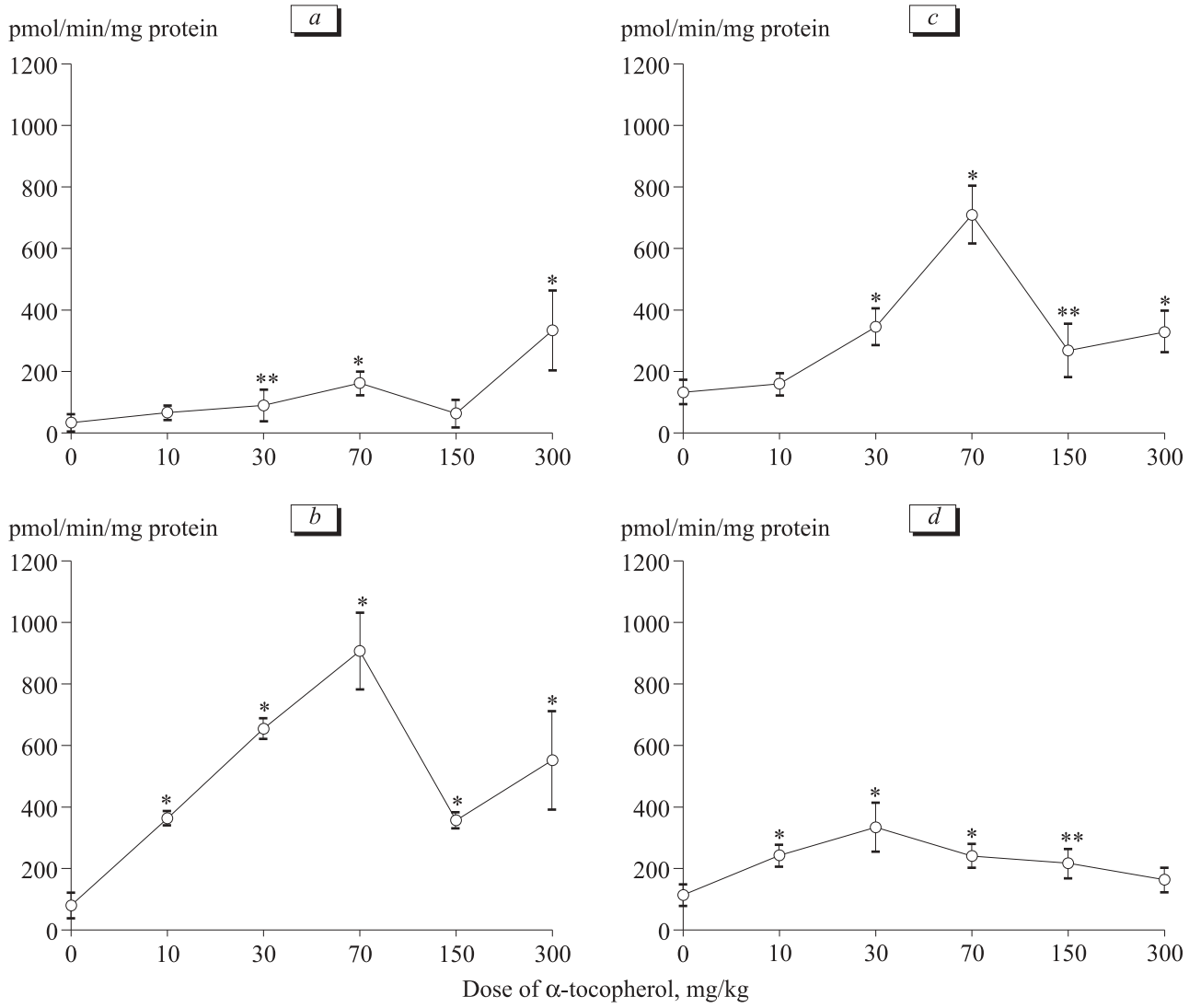

Fig. 3. Effect of α-tocopherol on activities of cytochromes P450, families 1 and 2. *a*) CYP2B1 (7-pentoxyresorufin O-dealkylation velocity); *b*) CYP2B+CYP2C (7-benzyloxyresorufin O-dealkylation velocity); *c*) CYP1A1 (7-ethoxyresorufin O-dealkylation velocity); *d*) CYP1A2 (7-methoxyresorufin O-dealkylation velocity). **p*<0.005, ***p*<0.05 compared to the control.

α-TP in doses of 30, 70, 150, and 300 mg/kg, respectively, in comparison with the control. 7-Methoxyresorufin-O-dealkylase activity characterizing CYP1A2 increased 2.1, 2.9, 2.1, and 1.9 times after treatment with α-TP in doses of 10, 30, 70, and 150 mg/kg, respectively, in comparison with the control (Fig. 3, *d*). Hence, the increase in the content and activity of phase I xenobiotic metabolism enzymes in the liver of experimental animals can be referred to molecular events regulated by α-TP. The total content of cytochrome P450 and activity of NADPH-cytochrome-P450-reductase in liver microsomes changed after α-TP treatment in a dose-dependent manner, the content of P450 increased after administration of α-TP treatment in dose of 10 mg/kg, while reductase activity increased only after treatment in a dose of 300 mg/kg (Fig. 1, *a*, *b*). Study of cytochrome P450 families 1A, 2B, and 2C showed that CYP1A1, CYP2B1, and CYP2B+CYP2C activities changed similarly depending on the dose of α-TP: they peaked at α-TP doses of 70 and 300 mg/kg and decreased at a dose of 150 mg/kg (Fig. 3, *a*, *b*, *c*). The increase in CYP1A2 activity was less pronounced and the peak was observed after treatment with α-TP in a dose of 30 mg/kg (Fig. 3, *d*).

The regulation of cytochrome P450 (*CYP*) genes can be realized at different levels including initiation of transcription and stabilization of mRNA and/or protein. Activity of cytochromes P450 can be limited by activity of NADPH-cytochrome-P450-reductase. In addition, activity of membrane-bound enzymes, such as cytochromes P450 and NADPH-cytochrome-P450-reductase, depends on lipid composition and physicochemical characteristics of membranes. The classical mechanism regulating genes belonging to the *CYP1* and

CYP2 families suggests participation of one of the three receptors: cytosol AhR (for *CYP1A1* and *CYP1A2* activation) and nuclear CAR and PXR (for *CYP2B* and *CYP2C* activation) [5]. In addition, the regulation of CYP1A2 can be realized at the level of mRNA stabilization.

Recent investigations on PXR knockout mice with PXR-specific agonists revealed AhR and AhR target genes (*CYP1A1* and *CYP1A2*) among the genes regulated with participation of PXR [9]. Another study provided the first evidence that all forms of vitamin E can activate PXR and the inducing efficiency of vitamin E depends on its concentration [7]. It is therefore probable that the increase of CYP1A1, CYP2B1, and CYP2C activities in the liver of rats treated with α-TP was a result of activation of transcription of the respective genes by a receptor-dependent mechanism. A drop of their activity at α-TP dose of 150 mg/kg can be explained by the rate-limiting role of NADPH-cytochrome-P450-reductase in monooxygenase reactions: when reductase activity increases at α-TP dose of 300 mg/kg, activities of these enzymes increase again. The increase in reductase activity, in turn, can be due to the stabilizing function of α-TP in microsomal membranes [12].

We should like to mention in conclusion, that α-TP induction of cytochromes P450 realizing drug and procarcinogene metabolism should be taken into consideration in clinical practice, because preventive treatment with vitamin E in high doses can modulate drug pharmacokinetics and lead to negative effects.

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