Inhibitory Effect of α-Tocopherol on Benzo(a)pyrene-Induced CYPA1 Activity in Rat Liver

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Combined treatment with benzo(a)pyrene (classic inductor of cytochromes P450 of subfamily 1A, CYP1A1 and CYP1A2) and α-tocopherol decreased benzo(a)pyrene-induced CYP1A1 activity in rat liver. Activities of CYP1A2, NADPH-cytochrome P450 reductase, and glutathione S-transferase remained unchanged under these conditions. Addition of α-tocopherol to benzo(a)pyrene-induced microsomes *in vitro* decreased activity of CYP1A1. Immunoblotting of proteins in liver microsomes with antibodies against CYP1A1 did not reveal differences in CYP1A1 protein content in the liver of rats receiving benzo(a)pyrene alone or in combination with α-tocopherol. The *in vivo* decrease in benzo(a)pyrene-induced CYP1A1 activity did not result from free radical-produced damage to CYP1A1. The inhibition of benzo(a)pyrene-induced CYP1A1 activity with α-tocopherol is probably realized at the posttranslational level.

Key Words: *α-tocopherol; benzo(a)pyrene; CYP1A; NADPH-cytochrome P450 reductase; glutathione S-transferase*

Enzymes for xenobiotic transformation play an important role in metabolism and bioactivation of exogenous compounds with different chemical composition (procarcinogens, drugs, environmental pollutants, *etc*.). Numerous forms of cytochrome P450 (CYP) and NADPH-cytochrome P450 reductase are the key phase I enzymes phase II enzymes include UDP-glucuronosyltransferase, glutathione S-transferase, N-acetyltransferase, sulfotransferase, and methyltransferase.

Subfamily 1A of cytochromes P450 (CYP1A) includes CYP1A1 and CYP1A2. They metabolize common environmental pollutants polycyclic aromatic carbohydrates (PAC) and arylamines. These cytochromes are involved in detoxification and activation of these compounds, which results in the formation of electrophilic metabolites binding to DNA [11]. CYP1A activity can increase under the influence of chemical compounds. PAC and halogenated aromatic carbohydrates of planar structure are classic inductors of CYP1A. Studies performed in the last decade revealed several compounds differing from aromatic carbohydrates by chemical structure, but capable of inducing CYP1A [6]. Previous experiments showed that α-tocopherol (α-TP) dose-dependently induces CYP1A in rat liver. However, α-TP is less potent than classic indictors [2].

Here we studied the effect of α-TP on CYP1A activity *in vivo* and *in vitro* induced by benzo(a)pyrene (BP). We showed that α-TP produced an inhibitory effect on BP-induced CYP1A1 activity. Possible mechanisms for this effect is discussed.

MATERIALS AND METHODS

Experiments were performed on 20 male Wistar rats weighing 100-120 g. The animals fed a standard diet. They were deprived of food for 1 day before killing. Experimental rats received 30% oil solution

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of α-TP (70 mg/kg perorally, 4 days), 1% oil solution of BP (25 mg/kg intramuscularly, 3 days), or α-TP and BP (starting from the 1st day of α-TP administration). Control animals did not receive test preparations. The animals were decapitated on day 5. The cytosolic fraction and microsomes of the liver were routinely isolated by differential centrifugation at 4°C. Protein content in microsomes and cytosol was measured by the method of Lowry using bovine serum albumin as standard [10]. Activities of CYP1A1 and CYP1A2 in liver microsomes were determined fluorometrically by the rate of O-dealkylation of highly specific substrates 7-ethoxy- and 7-methoxyresorufins, respectively [5]. Activity of NADPH-cytochrome P450 reductase in liver microsomes was estimated by the rate of cytochrome *c* reduction [13]. Cytosolic glutathione S-transferase activity was evaluated by the rate of 2,4-dinitrophenyl glutathione formation. 1-Chloro-2,4-dinitrobenzene and S-2,4-dinitrophenyl glutathione served as the glutathione acceptor and standard, respectively [8]. For detection of CYP1A1 and CYP1A2 apoenzymes, microsomal proteins were separated by disc electrophoresis, placed on a nitrocellulose membrane, and incubated with monoclonal mouse antibodies against rat CYP1A1 and CYP1A2 (clone 14H5) [15]. Immunoreactive proteins were visualized with ExtrAvidin alkaline phosphatase staining kit (Sigma) according to manufacturer's instruction. The intensity of lipid peroxidation (LPO) in rat liver microsomes was estimated by the amount of malonic dialdehyde (MDA) in the reaction with thiobarbituric acid [1]. Nonenzymatic LPO was activated as described elsewhere [7]. Ascorbate was added to a final concentration of 1 mM [7]. The solution of α-TP in dimethylsulfoxide (final concentration 17.7-176.7 μM) was *in vitro* added to microsomes immediately before measurement of 7-ethoxyresorufin activity. Activity of 7-ethoxyresorufins was measured 3 times for each concentration of α-TP. Reference activity was determined after each 6 measurements.

The results were analyzed by Students' *t* test and Mann—Whitney *U* test (Statistica 5.5 software).

RESULTS

Table 1 represents the data on activity of enzymes for xenobiotic biotransformation in the liver of control rats and animals receiving α-TP and/or BP. α-TP increased activities of CYP1A1 and CYP1A2 by 5.4 and 2.1 times, respectively, compared to the control. Activities of NADPH-cytochrome P450 reductase and glutathione S-transferase remained unchanged under these conditions. However, the mean value of enzyme activity in experimental rats was above the control. Administration of BP considerably increased activities of CYP1A1, CYP1A2, and glutathione S-transferase (by 187.8, 14.6, and 1.8 times, respectively, compared to the control). NADPH-cytochrome P450 reductase activity remained unchanged under these conditions. Combined treatment with α-TP and BP 1.3-fold decreased CYP1A1 activity compared to rats receiving BP. Activities of CYP1A2, glutathione S-transferase, and NADPH-cytochrome P450 reductase did not differ in animals receiving BP alone or in combination with α-TP.

α-TP can exhibit antioxidant and prooxidant properties under different conditions *in vivo* [4]. Free radicals damage cytochrome P450, which is accompanied by a decrease in its activity [12]. The content of MDA (LPO product) in rat liver microsomes was measured to evaluate the mechanism of the inhibitory effect of α-TP on BP-induced CYP1A1 activity. MDA content decreased in rats receiving α-TP alone or in combination with BP (by 6.4 and 3.4 times, respectively). Therefore, α-TP in the specified dose exhibited high antioxidant activity (Table 1).

TABLE 1. Activity of Enzymes for Xenobiotic Transformation and MDA Content in Rat Liver *in Vivo* (*M*±*m*)

Group	Enzyme				MDA content, nmol/mg protein
	CYP1A1 (7-ethoxyresorufins), nmol/mg protein/min	CYP1A2 (7-methoxyresorufins), nmol/mg protein/min	Glutathione S-transferase, nmol/mg protein/min	NADPH-cytochrome P450 reductase, nmol/mg protein/min	
Control	0.1320±0.0208	0.114±0.019	812.0±64.3	51.00±10.43	82.90±14.46
α-TP	0.7090±0.0756*	0.2410±0.0214*	1015±56	74.01±9.53	13.00±5.79*
BP	24.80±1.52*	1.670±0.242*	1458.0±230.5*	51.10±3.78	85.40±11.97
BP+α-TP	18.60±1.64*+	1.580±0.208*	1136.0±112.8*	49.90±4.12	25.10±5.51*+

Note. $p<0.05$: *compared to the control group; +compared to the BP group.

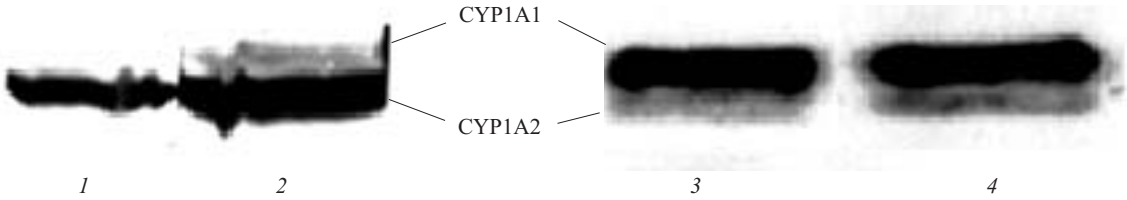

Fig. 1. Immunoblotting of proteins in rat liver microsomes with monoclonal antibodies against CYP1A1 and CYP1A2. Liver microsomes from control rats (*1*); liver microsomes from animals receiving α-tocopherol (α-TP, *2*); liver microsomes from animals receiving BP (*3*); liver microsomes from animals receiving BP and α-TP (*4*). Bands *1* and *2*: 80 μg total microsomal protein; bands *3* and *4*: 0.5 μg total microsomal protein.

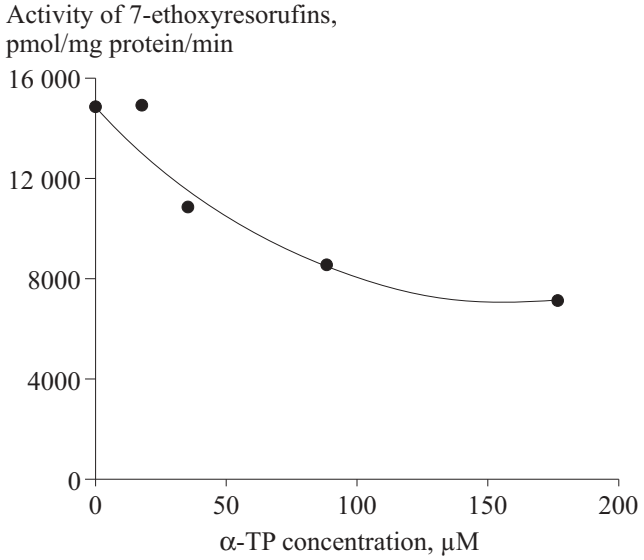

Fig. 2. Inhibition of CYP1A1 in BP-induced rat liver microsomes by α-TP. Each point on the curve represents the mean of 3 independent measurements.

For evaluation of the mechanism of *in vivo* inhibitory effect of α-TP on BP-induced CYP1A1 activity, the contents of CYP1A1 and CYP1A2 apoenzymes in rat liver microsomes were measured by the immunoblotting technique. CYP1A1 apoenzyme was detected in the liver of rats receiving α-TP and/or BP (Fig. 1). Liver microsomes from rats receiving BP alone or in combination with BP did not differ in the intensity of CYP1A1 apoenzyme bands. Therefore, the inhibitory effect of α-TP on BP-induced CYP1A1 activity is realized at the post-translational level.

Figure 2 illustrates the *in vitro* effect of α-TP on CYP1A1 activity in BP-induced rat liver microsomes. Addition of α-TP to BP-induced rat liver microsomes dose-dependently decreased CYP1A1 activity.

We studied the *in vitro* effect of α-TP in CYP1A1 activity in rat liver microsomes during induction of ascorbate-dependent LPO. The ratio of CYP1A1 activity remaining after induction of ascorbate-dependent LPO by BP alone or in combination with α-TP was 10.50±4.16 and 89.40±2.91%, respectively ($p<0.05$). CYP1A1 activity in samples incubated without ascorbate was taken as 100%. CYP1A1 activity in liver microsomes from BP-treated rats decreased by 9.5 times during induction of ascorbate-dependent LPO. Under these conditions CYP1A1 activity in liver microsomes from animals receiving BP and α-TP did not differ from that in samples not containing ascorbate.

Our results indicate that α-TP *in vivo* decreases BP-induced CYP1A1 activity, which is realized at the posttranslational level and does not depend on the progression of free radical processes. α-TP has no effect on activity of NADPH-cytochrome P450 reductase. Therefore, suppression of CYP1A1 is not associated with the rate-limiting component of monooxygenase reactions [14]. Probably, the effect of α-TP on BP-induced CYP1A1 activity is related to inhibition of this enzyme (as shown in *in vitro* experiments).

Several weak inductors of cytochromes P450 can decrease CYP1A activity induced by classic indictors [9]. Our findings provide support to these data. α-TP induces CYP1A1 activity in the liver of intact animals, but inhibits this enzyme in the liver of BP-treated specimens. Different effects of PAC are realized via activation of CYP1A1 [3]. The revealed properties of α-TP can be used to prevent adverse effect of PAC on the organism. α-TP does not modulate BP-induced activity of glutathione S-transferase. The enzyme catalyzes phase II of xenobiotic transformation and converts toxic products into safe compounds. These data indicate that α-TP can prevent the adverse effect of PAC on the organism.

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