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Transcriptional Activation of Cytochrome P450 1A1 with α-Tocopherol

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Peroral administration of α-tocopherol in a daily dose of 150 mg/kg for 1, 4, 8, and 12 days leads to induction of cytochromes P450 1A in male rats. Activity of CYP1A1 and CYP1A2 increased most significantly one day after α-tocopherol administration (by 2.6 and 2.7 times, respectively). CYP1A1 was immunohistochemically detected in rat liver microsomes during this period. The content of *CYP1A1* mRNA significantly increased in the liver. The amount of *CYP1A2* mRNA and regulatory proteins for signal activation of CYP1A1 (*AhR* and *Arnt*) remained unchanged after treatment with α-tocopherol.

Key Words: *α-tocopherol; CYP1A1; CYP1A2; AhR; Arnt*

Cytochromes P450 (CYP) of subfamily 1A are presented by CYP1A1 and CYP1A2. They catalyze detoxification and toxification of polycyclic aromatic carbohydrates and arylamines. CYP1A are inducible enzymes. The content of these enzymes in tissues increases several hundred times under the influence of various inductors, including polycyclic aromatic carbohydrates and chlorinated *p*-dibenzodioxins [13]. Transcriptional Ah-receptor-dependent (AhR) signal transduction is the only known pathway for *CYP1A* activation. Before binding to the inducing ligand, cytoplasmic AhR is associated with heat shock protein hsp90. Binding of the ligand induces translocation of AhR into the nucleus and dimerization with Arnt protein. This complex gains affinity for specific sequences of the DNA enhancer. Binding to the enhancer is accompanied by disruption of nucleosomal structure in the promoter and activation of gene transcription [13]. Recent studies showed that *CYP1A1* can be activated not only by standard inducing ligands of AhR, but also by some compounds structurally differing from polycyclic aromatic substances [9].

Widely used antioxidant α-tocopherol (AT), the most active form of vitamin E, modulates CYP1A activity in rat liver [1]. The mechanisms for AT-produced changes not associated with its antioxidant function are poorly understood. Published data show that AT produces a variety of posttranscriptional and transcriptional effects [8].

To evaluate the mechanism for CYP1A induction with AT, we assayed temporal changes in activity of CYP1A1 and CYP1A2 and studied the effect of AT on expression of genes encoding *CYP1A* and regulatory proteins *AhR* and *Arnt*.

MATERIALS AND METHODS

Experiments were performed on 25 male Wistar weighing 100-120 g. The animals fed a standard laboratory diet and were deprived of food 1 day before euthanasia. Experimental rats perorally received 30% oil solution of AT in a daily dose of 150 mg/kg for 1, 4, 8, and 12 days. The animals were narcotized and killed by decapitation.

Liver microsomes were isolated by differential centrifugation at 4°C. Total protein content, total concen-

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tration of cytochrome P450, and activity of NADPH-cytochrome *c* reductase were measured in rat liver microsomes [1]. O-Dealkylation of substrates highly specific for rat CYP1A1 and CYP1A2 (7-ethoxy- and 7-methoxyresorufins, respectively) was assayed fluorometrically in liver microsomes by the rate of resorufin formation [2]. CYP1A1 was detected immunohistochemically. Microsomal proteins (80 mg) were separated by disc electrophoresis, transferred onto a nitrocellulose membrane [12], and incubated with monoclonal mouse antibodies against rat CYP1A1 a CYP1A2 (clone 14H5). Immunoreactive proteins were visualized with ExtrAvidin Alkaline Phosphatase Staining Kit (Sigma).

Total liver cell RNA was isolated by the SDS-phenol method [3]. The content of specific mRNAs for *CYP1A1*, *CYP1A2*, *AhR*, and *Arnt* was determined in a semiquantitative reverse transcription polymerase chain reaction (RT-PCR) [7]. *In vitro* synthesis of cDNA was performed using 400 ng RNA per sample. Amplification involved pairs of primers synthesized on an ASM-800 device (Biosset). Primers were specific for nucleotide sequences of *CYP1A1* (direct, 5'-CTGGTTCTGGATACCCAGCTG-3', reverse, 5'-CCTAGGGTTGGTTACCAGG-3'), *CYP1A2* (direct, 5'-CTGGTTCTGGATACCCAGCTG-3', reverse, 5'-CCTAGGGTTGGTTACCAGG-3'), *AhR* (direct, 5'-TCCATGTACCAGTGCCAGG-3'; reverse, 5'-ATATCAGGAAGAGGCTGGGC-3'), *Arnt* (direct, 5'-GTCTCCCTCCCAGATGATGA-3'; reverse, 5'-AAGAGCTCCTGTGGCTGGTA-3'), and β-actin (internal standard; direct, 5'-CGTTGACATCCGTAAAGACCTCTA-3'; reverse, 5'-TAAAACGCAGCTCAGTAACAGTCCG-3'). PCR was performed in the following stages: de-

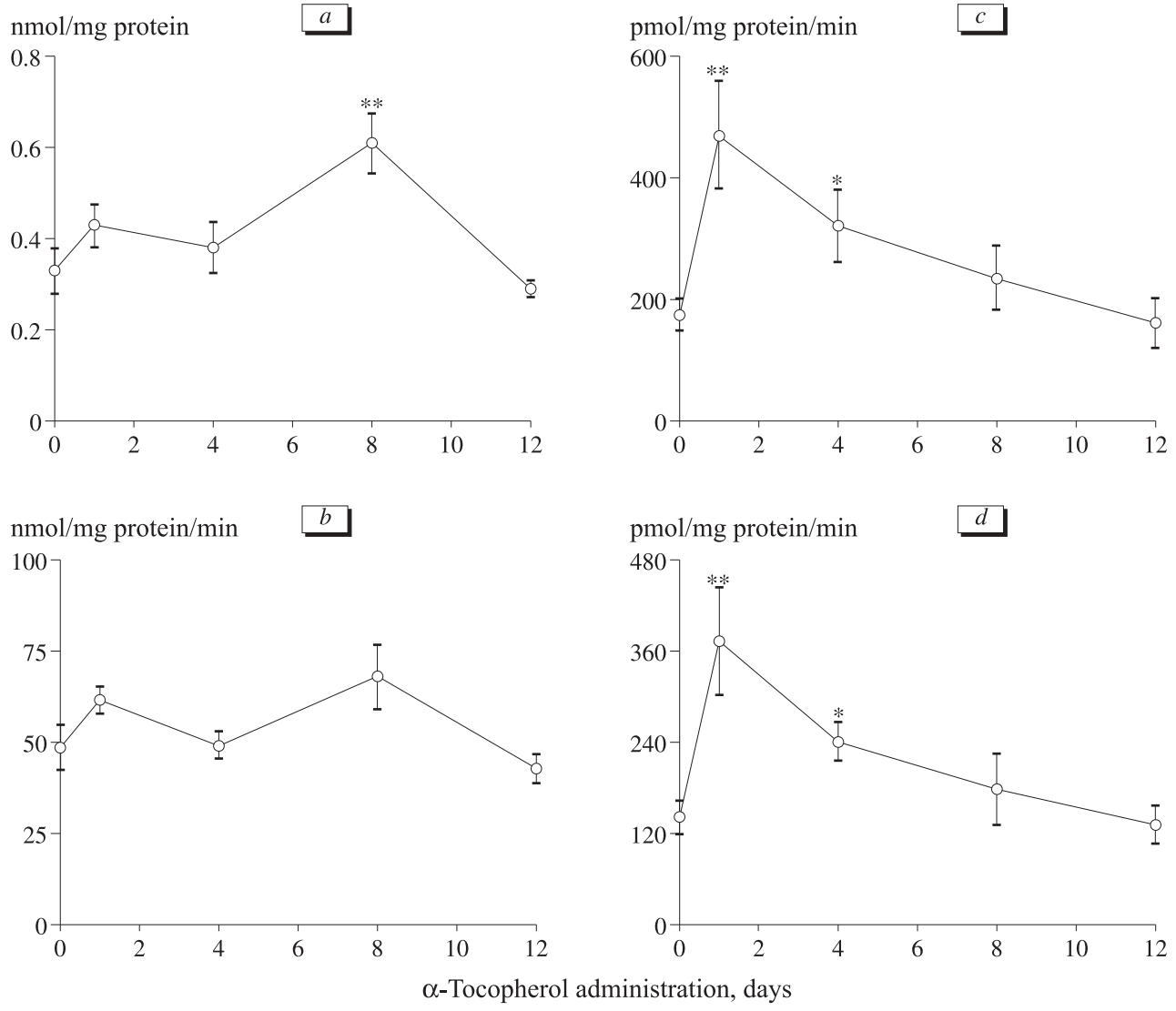

Fig. 1. Total content of cytochromes P450 (*a*) and activities of NADPH-cytochrome *c* reductase (*b*), CYP1A1 (*c*), and CYP1A2 (*d*) after administration of α-tocopherol. Ordinate: cytochrome P450 content per 1 mg microsomal protein (*a*), rate of cytochrome *c* oxidation (*b*), and rate of resorufin formation per 1 mg protein over 1 min (*c*, *d*). **p*<0.05 and ***p*<0.01 compared to the baseline level.

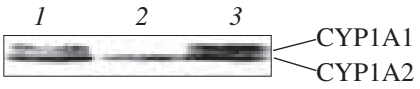

Fig. 2. Immunoblotting of proteins in rat liver microsomes with monoclonal antibodies (clone 14H5) against CYP1A1/A2: α-tocopherol, 1 day (80 μg protein, *1*); control (80 μg protein, *2*); and 3-methylcholanthrene (11 μg protein, *3*).

naturation (95°C, 3 min: *CYP1A1/A2*; 94°C, 3 min: *AhR* and *Arnt*; 94°C, 1 min: β-actin); amplification with 35 (*CYP1A1*, *AhR*, and *Arnt*), 29 (*CYP1A2*), or 28 cycles (β-actin); denaturation at 94°C for 30 sec; annealing at 56 (*CYP1A1*), 60 (*CYP1A2*), and 57°C for 1 min (*Ahr*, *Arnt*) or at 65°C for 30 sec (β-actin); elongation at 72°C for 1 min; and final elongation at 72°C for 4 min. Each sample was amplified 2 times. PCR products were assayed after electrophoresis in 2% agarose gel, ethidium bromide staining, and scanning on a DNA Analyzer system. Densitometry was performed using Total Lab software. Gene mRNA content was estimated by the ratio between optical density of the RT-PCR product obtained with specific primers for gene sequences and optical density of the product isolated with specific primers for the β-actin gene.

The results were analyzed by Students' *t* test and Mann—Whitney *U* test.

RESULTS

The total content of cytochromes P450 increased most significantly on day 8 of treatment with AT (by 1.8 times, Fig. 1, *a*). NADPH-cytochrome *c* reductase activity remained practically unchanged. In rats receiving AT, catalytic activities of CYP1A1 and CYP1A2 increased most significantly on day 1 (by 2.7 and 2.6 times, respectively) and remained above the baseline level after 4 days (by 1.8 and 1.7 times, respectively). Activities of CYP1A1 and CYP1A2 practically did not differ on day 8 of AT administration and returned to normal by the 12th day.

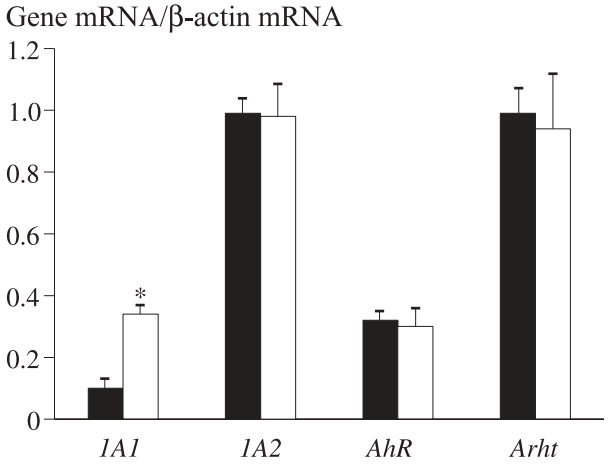

Fig. 3. Contents of mRNA for *CYP1A1*, *CYP1A2*, *Ahr*, and *Arnt* in the liver of control rats (dark bars) and animals receiving α-tocopherol for 1 day (light bars). *$p<0.01$ compared to the control.

Our results show that the content of cytochromes P450 and activities of CYP1A1 and CYP1A2 reached maximum in various periods of treatment with AT. Therefore, the increase in total P450 content is related to changes in the amount of other cytochromes whose induction depends on the influence of AT (*e.g.*, CYP2B/2C) [1]. It should be emphasized that AT is a weak inductor of CYP1A. AT is less potent than benzopyrene and 3-methylcholanthrene in inducing CYP1A. These classic inductors increase CYP1A activity more than by 100 times. Previous studies of time and dose dependences showed that AT increases CYP1A activity not more than by 2-5 times [1].

Apoenzyme CYP1A was not detected in microsomes from control rats. However, this substance was present in rat liver microsomes on day 1 of treatment with AT (Fig. 2). The amount of microsomes from AT-receiving rats applied on the gel track was 80 times higher than that of benzopyrene-induced microsomes. These data illustrate differences in the degree of induction.

Single administration of AT 3.4-fold increased the content of *CYP1A1* mRNA in rat liver, but had no effect on the expression of *CYP1A2*. AT did not modulate expression of genes for AhR and Arnt involved in activation of CYP1A (Fig. 3).

AT induces transcription of CYP1A1, which suggests that this substance can activate AhR. No correlation was revealed between catalytic activation of CYP1A2 and increase in the content of mRNA for the corresponding gene. It can be hypothesized that this enzyme is activated at the posttranscriptional level. The possibility for posttranscriptional modification of CYP1A2 activity was reported previously [11].

Previous studies showed that classic inductors 3-methylcholanthrene and tetrachlorodibenzodioxin increase expression of genes for regulatory proteins AhR and Arnt [6,10,14]. Moreover, CYP1A activity in cultured cells increases after introduction of plasmids expressing AhR and Arnt [10]. AT is a less potent inductor of CYP1A than other classic inductors. It can be related to a limiting role of regulatory proteins whose expression is not induced by AT.

The induction of CYP1A was maximum after single administration of AT. AT possesses lipophilic properties and can accumulate in the organism. However, further treatment with AT did not increase and even decreased CYP1A activity (compared to day 1). Published data show that CYP1A1 activity increases after administration of AT in doses of 10-70 mg/kg [1], but decreases after treatment with higher doses of AT, despite the increase in total tocopherol content. These data suggest that the increase in the dose and/

or time of treatment with AT triggers the mechanism of negative AhR-dependent regulation of CYP1A1. Phosphorylation of phosphoproteins Arnt and AhR is probably required for dimerization and binding to DNA, respectively [13]. On the one hand protein kinase C is involved in AhR-mediated signal transduction [4,5]. On the other hand, AT can inhibit protein kinase C [8]. Probably, the increase in liver AT content after repeated treatment or administration of the compound in high doses reduces the degree of CYP1A1 induction due to a decrease in the amount of phosphorylated AhR.

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