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Correction of Biotransformation of Xenobiotics by α-Tocopherol in Combination with Nicotinamide and Methionine in the Liver Damaged by Ultrasound

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Six day after rat liver sonication, the content of cytochrome P-450, rate of NADPH oxidation, activity of NADPH—cytochrome P-450 reductase, and rate of aniline hydroxylation in the microsomal fraction decrease. After 12 days, the rate of ethylmorphine N-demethylation also decreases. Intragastral administration of methionine, nicotinamide, and vitamin E for 6 and 12 days activates these enzymes and uridine 5'-diphosphate glucuronyl transferase.

Key Words: *ultrasound damage to the liver; biotransformation of xenobiotics; α-tocopherol; nicotinamide; methionine*

"Loosening" of the lipoprotein complex of cellular and subcellular membranes caused by ultrasound leads to an increase in membrane permeability and inhibition of membrane-bound enzymes, as shown in experiments with mitochondria. This effect has been related to cavitation and free-radical processes [5,6]. In the present study the effect of ultrasound on the activity of monoxidases and glutathione and glucuronyl transferases and the protective effect of the membrane-stabilizing complex consisting of α-tocopherol, nicotinamide, and methionine were examined.

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MATERIALS AND METHODS

Experiments were performed on 32 outbred male rats weighing 180-200 g. The liver was sonicated (2 W/cm^2) after laparotomy under ether anesthesia. α-Tocopherol (50 mg/kg) in combination with nicotinamide (50 mg/kg) and methionine (200 mg/kg) was administered intragastrally in starch gel for 6 and 12 days. Control rats (laparotomy) were given the same volume of starch gel. The contents of cytochromes P-450 and b_5, activities of NADPH—cytochrome P-450

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and NADPH—cytochrome b_5 reductases (potassium ferricyanide employed as an electron acceptor), rates of NADPH and NADH oxidation, N-demethylation of ethylmorphine and amidopyrine, and p-hydroxylation of aniline, and the contents of uridine 5'-diphosphate (UDP) glucuronyl transferase, glutathione S-transferase, and protein were determined in the microsomal fraction [2]. The degree of "loosening" (permeability) of microsomal membranes was assessed by the intensity of fluorescence of the lipophilic probe 1-anilinonaphthalene-8-sulfonate (ANS^-) [4]. The *in vivo* activity of the glucuronyl-conjugating system was determined by the rate of urinary excretion of free and conjugated glucuronic acid [9].

RESULTS

On day 6 after sonication, the intensity of ANS^- (10 μl in microsome suspension containing 0.1 mg protein/ml) increased by 36% compared with the control (Table 1). Analysis of the fluorescence in relation to the concentration of the probe and microsomal membranes showed that the number of ANS^- binding sites increased, indicating a higher accessibility of hydrophobic membrane components to the probe as a result of ultrasound damage. The content of cytochrome P-450, activity of NADPH—cytochrome P-450 reductase, and rate of NADPH oxidation decreased by 40, 16, and 28%, respectively. The content of cytochrome b_5 remained unchanged, while the activity of NADH—cytochrome b_5 reductase and the rate of NADH oxidation tended to decrease. A decrease in the rate of substrate oxidation was most pronounced with aniline (by 42%). The activity of glucuronyl and glutathione transferases, enzymes involved in the second stage of xenobiotic biotransformation, remained practically unchanged in the microsomal fraction of sonicated liver. However, a tendency toward an increase in urinary excretion of both total and conjugated glucuronic acid was observed.

Judging from the intensity of ANS^- fluorescence on day 12 after liver sonication, the permeability of microsomal membranes is restored without reaching the initial level. The cytochrome P-450 content, rate of NADPH oxidation, and activities of ethylmorphine N-demethylase and aniline hydroxylase were decreased by 45, 25, 59, and 53%, respectively. The activities of glutathione and glucuronyl transferases remained at the control level.

Administration of α-tocopherol in combination with nicotinamide and methionine facilitated the restoration of membrane structure and enzyme activities. On day 6 of the treatment, the intensity of ANS^- decreased, while the activity of NADPH—cytochrome P-450 reductase increased to the control level. The activities of NADPH oxidase and glucuronyl transferase were, respectively, 46 and 34% higher than in the control. Urinary excretion of free and conjugated glucuronic acid increased by 65 and 42%, respectively. The cytochrome P-450 content and the rate of aniline hydroxylation decreased by 34 and 53%.

On day 12 of the treatment, the content of cytochrome P-450 and activities of NADPH oxidase, NADH—cytochrome b_5 reductase and ethylmorphine N-demethylase were practically the same as in the control, while the content of cytochrome b_5 and activities of NADH oxidase, NADPH—cytochrome P-450 reductase, amidopyrine N-demethylase, glutathione and UDP-glucuronyl transferase were, respectively, 44, 134, 24, 83, 46, and 112% higher than in the control. The activity of cytochrome P-450IIE1-dependent aniline hydroxylase was decreased.

Thus, sonication (2 W/cm^2, 1 min) of the liver *in vivo* leads to destruction of the endoplasmic reticulum membranes with denudation of their hydrophobic components, as evidenced by increased intensify of ANS^- fluorescence, and a considerable decrease in the activity of the membrane-bound monoxidase system, predominantly NADPH-dependent and cytochrome P-450-containing hydroxylation chain. Cytochrome P-450, which is located in the hydrophobic zone of microsomal membranes [1], is strongly inhibited, while NADPH—cytochrome P-450 reductase, which is located on the outer hydrophilic layer, is inhibited to a lesser extent. NADH-dependent electron-transporting chain (with cytochrome b_5 acting as an active center) is located in the hydrophilic zone and remains practically undamaged after sonication. The same is true for glutathione and UDP-glucuronyl transferases, which are located on the inner membrane surface [8]. From these findings it can be hypothesized that inhibition of cytochrome P-450-dependent hydroxylation system caused by ultrasound is due predominantly to structural changes in lipid microenvironment of cytochrome P-450 induced by free-radical processes rather than mechanical destruction. This hypothesis is supported by the observation that the α-tocopherol—nicotinamide—methionine complex with pronounced antioxidant activity [3,7] prevents inhibition of microsomal monoxidases. Protective effect of this complex on the monoxidase system upon ultrasound irradiation of the liver and an increase in the activity of membrane-bound UDP-glucuronyl and glutathione transferases may be due to the membrane-stabilizing activity of α-tocopherol [3], electron-donor properties of nicotinamide (NADH and NADPH), and ability of methionine to stimulate restoration of membrane structure by increasing phospholipid and protein production.

TABLE 1. Effect of Intragastral Administration of a-Tocopherol (50 mg/kg) in Combination with Nicotinamide (50 mg/kg) and Methionine (200 mg/kg) on ANS^- Fluorescence, Activity of Monoxygenase, and Glucuronyl and Glutathione-Conjugating Systems of Rat Liver Microsomes After Liver Sonication (2 W/cm², 1 min) ($M \pm m$, n=8)

Parameter	Day 6			Day 12		
	control	ultrasound	ultrasound+ α-tocopherol, nicotinamide, and methionine	control	ultrasound	ultrasound+ α-tocopherol, nicotinamide, and methionine
ANS^- fluorescence, rel. units (10 μmol/0.1 mg protein/ml	73.31±4.12	99.85±4.20 136	85.22±0.04 116 85*	69.53±6.01	81.33±5.00 117	75.65±6.11 109 93
Cytochrome P-450, nmol/mg	0.65±0.05	0.39±0.12 60*	0.43±0.09 66* 110	0.64±0.10	0.35±0.13 55*	0.45±0 11 70 129
NADPH—cytochrome P-450 reductase, μmol/min/kg	0.25±0.02	0.21±0.01 84*	0.27±0.01 108 129*	0.25±0.01	0.22±0.01 88	0.31±0.02 124* 141*
NADPH oxidation, nmol/min/ml	3.99±0.25	2.86±0.05 72*	5.81±0.41 146* 203*	4.56±0.30	3.43±0.32 75*	5.37±0.51 118 157*
Cytochrome b_5, nmol/mg	0.46±0.02	0.46±0.04 100	0.47±0.02 102 102	0.45±0.03	0.44±0.03 98	0.65±0.05 144* 148*
NADH—cytochrome b_5 reductase, μmol/min/mg	6.88±0.30	5.84±0.43 85	5.51±0.21 80* 94	6.04±0.26	4.94±0.33 82	6.32±0.32 105 128*
NADH oxidation, nmol/min/mg	6.91±0.72	6.18±0.23 89	5.68±0.51 82 92	2.48±0.24	2.56±0.31 103	5.81±0.79 234* 227*
N-demethylation of amidopyrine, nmol/min/mg	10.85±0.24	10.18±0.25 94	10.25±0.74 95 101	5.73±0.67	6.53±0.35 114	10.52±0.65 184* 161*
N-demethylation of ethylmorphine, nmol/min/mg	11.82±0.61	9.12±1.48 77	12.27±1.36 104 135	8.66±1.72	3.56±0.32 41*	12.35±1.16 143 347*
p-Hydroxylation of aniline, nmol/min/mg	0.45±0.05	0.26±0.04 58*	0.21±0.02 47* 81	0.47±0.10	0.22±0.03 47*	0.23±0.02 49* 105
Microsomal glutathione S-transferase, μmol CDB/min/mg	0.11±0.008	0.11±0.01 100	0.09±0.009 82 82	0.11±0.007	0.12±0.01 109	0.16±0.01 146* 133*
UDP-glucuronyl transferase, nmol PNP/min/mg	4.67±0.28	4.58±0.38 98	6.24±0.38 134* 136*	3.85±0.46	4.08±0.38 106	8.16±0.69 212* 200*
Glucuronic acid, total mg per sample	2.41±0.13	3.13±0.31 130	3.98±0.59 165* 127	3.03±0.21	3.58±0.38 118	3.19±0.40 105 89
conjugated	1.94±0.16	2.39±0.22 123	2.76±0.20 142* 116	2.19±0.19	2.99±0.37 137	2.37±0.31 108 79

Note. Value above the line is percent of changes compared with the control, value below the line is percent of changes compared with that after sonication. CDB: 1-chloro-2,4-dinitrobenzene, PNP: p-nitrophenol. *p<0.05 compared with the control.

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