Content and Redistribution of Vitamin E in Tissues of Wistar Rats Under Oxidative Stress Induced by Hydrazine


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Abstract. Hydrazine toxicity is associated with generation of several kinds of free radicals and oxidative stress in cell. Experiments in vivo have demonstrated that oxidative stress could either diminish or increase concentration of vitamin E in some tissues. Thus in the present study we performed experiments to determine whether hydrazine-induced oxidative stress would change the tissue levels of the vitamin. Seven days of hydrazine intoxication led to accumulation of different amounts of vitamin E: 215% in the liver, 118% in the heart, 135% in the spleen, and 100% in the muscle over control value. There were no changes in the level of the vitamin in kidney and pancreas, despite its significant depletion in the serum. In tissue that accumulated vitamin E after hydrazine treatment, an increased of oxidative stress measured by the concentration of lipid-soluble fluorophore was observed. Significant increases of 107%, 46%, 72%, and 58% over control values were observed in the liver, heart, spleen, and muscle, respectively. Rats treated with hydrazine and pharmacological doses of α-tocopherol accumulated higher concentrations of vitamin E in all studied tissues compared with the α-tocopherol-only treated rats. However, in tissues with elevated levels of fluorophore as liver, heart, spleen, and muscle, the accumulation of vitamin E was 5.03, 4.5, 4.03, and 4.6 times higher than in α-tocopherol-treated rats, respectively. Vitamin E concentration was much higher than in kidney and pancreas, where the accumulation was only 2.31 and 2.6 times higher. On the other hand, 3 days of hydrazine treatment did not change either the level of lipid-soluble fluorophore or the level of vitamin E in the liver mitochondria, microsomes, and homogenate. In skeletal muscle vitamin E caused decreased lipofuscin accumulation, and in pancreas vitamin E increased lipofuscin accumulation. Our data indicate that hydrazine is able to modify significantly vitamin E status in different rat tissues.

Vitamin E is a lipid-soluble molecule that exerts its action mainly in biological membranes, protecting them from damage. The function of vitamin E is connected with its wide range of properties. One of the main properties of vitamin E is its antioxidative action: it can react with peroxyl, alkoxyl, and superoxide radicals as well as physical quenching of singlet oxygen (Halliwell and Gutteridge 1989).

The scavenging activity of vitamin E allows it to protect unsaturated lipids of biological membranes from the oxidation caused by free radical species. Moreover, it has been recently demonstrated that membrane proteins can also be protected from oxidation by vitamin E (Takenaka et al. 1991). Vitamin E is also able to protect biological membranes from phospholipases and products of their action, free fatty acids and lysophospholipids (Kagan 1989).

In vivo experiments on animals have demonstrated that vitamin E decreases the toxicity of several compounds, including nitrogen dioxide (Elsayed and Mustafa 1982), hydrazine (Antosiewicz et al. 1994a), and methyl ethyl ketone peroxide (MEKP) (Ando and Tappel 1985). Moreover, it has been observed that vitamin E treatment can diminish carcinogenic properties of some toxins (Cook and McNamara 1980). On the other hand, increased susceptibility to cigarette smoke was observed in rats maintained on a basal vitamin E–deficient diet (Chow et al. 1984). Because of these properties, great attention has been focused on the status of vitamin E in tissues.

Some pathological conditions can influence distribution of vitamin E in an organism. Decreased levels of vitamin E in plasma and an increased in lungs were observed after oxidative stress induced by tobacco smoke (Chow et al. 1989). Similar phenomena were observed in the pancreas during acute pancreatitis (Antosiewicz et al. 1995). However, oxidative stress induced by MEKP lowers the level of vitamin E both in blood and liver (Warren and Reed 1991). Decreased vitamin E content in muscle was observed after endurance training, which also is accompanied by oxidative stress (Packer et al. 1989). Intoxication by 1,2-dibromomethane, which is not connected with oxidative stress, led to depletion of hepatic vitamin E levels but at the same time an elevation of the level in plasma (Warren et al. 1991). An increase of vitamin E content in the heart and simultaneously a decrease in the liver was observed in rats following dietary fatty acid manipulation (Chautan et al. 1990). An increase of linoleate content in adipose tissue led to a decrease in the content of vitamin E in the plasma (Witting...
in the tissues of aged animals, changes in lipid composition (Barret and Horton 1975), increased lipid peroxidation (Uysal et al. 1989) and accumulation of vitamin E (Weglicki et al. 1969) were observed. It seems clear from the literature that the modification of vitamin E status in tissues can be influenced by such factors as oxidative stress and tissue lipid composition. Hydrazine and its derivatives are used in industry as well as in medicine. Some drugs used for treatment of tuberculosis and hypertension are derivatives of hydrazine. However, hydrazine and its derivatives possess a toxic activity that is connected with the generation of different kinds of free radicals. Most of the radical species are generated from hydrazine at the level of cytochrome P450 (Noda et al. 1987) in the liver. Hydrazine-derived free radicals can induce oxidative stress (Antosiewicz et al. 1994a).

In the present study we use hydrazine as a toxin, which both induces oxidative stress and changes fatty acids composition in the liver (Wakabayashi et al. 1987), to investigate its effect on vitamin E distribution in several rat tissues. The experiments presented here were designed to address three major questions: (1) Does hydrazine-induced oxidative stress influence the level of vitamin E in a similar manner to oxidative stress caused by tobacco smoke in lungs or that induced by MEKP in the liver? (2) Is the accumulation or depletion of vitamin E caused by oxidative stress tissue-specific? (3) Can pharmacological doses of vitamin E protect rats from hydrazine toxicity? Our results indicate that hydrazine induces accumulation of vitamin E in liver, heart, spleen, and muscle but decreases its concentration in the serum. We also observed antioxidant and pro-oxidant activity of vitamin E.

Materials and Methods

Materials
dl-α-Tocopherol acetate was purchased from Sigma Chemical (St. Louis); dl-α-tocopherol was purchased from Wako Pure Chemical Industries, (Osaka, Japan). Hydrazine dichloride and quinine were obtained from Kanto Chemical. Hexane for spectroscopy and ethanol for trace analysis were from Cica-Merck. All other chemicals were of analytical grade.

Male Wistar rats, 4 weeks of age, were used in the present study. The animals were divided into four groups. Control animals (C) were fed a powdered chow diet, a hydrazine group (Hz) received diet containing 0.5% hydrazine dichloride for 7 or 3 days, the vitamin E group (E) received the normal diet and daily 700 mg per kg body weight dl-α-tocopherol diacetate intraperitoneally, and the fourth group (HzE) was treated with 700 mg α-tocopherol per kg body weight plus 0.5% hydrazine in the diet. The animals in the E and HzE groups were treated with vitamin E for 10 days. In HzE group vitamin E was administrated 3 days before the hydrazine.

Preparation of the Tissue Homogenates

The tissues, after washing and removing connecting tissues, were weighed and cut into small pieces. Homogenates (10% w/v) were prepared in the solution containing 50 mM phosphate buffer, pH 7.3, and 0.5 mM EDTA.

Isolation of Mitochondria from Rat Liver

Mitochondria were isolated essentially as reported before (Wakabayashi et al. 1979). Isolation medium contained 2 mM Hepes (N-hydroxyethyl piperazine-N-2-ethanesulfonic acid), pH 7.4, 70 mM sucrose, 220 mM mannitol, 0.05% bovine serum albumin fatty acid-free (Sigma), and 0.1 mM EDTA.

Vitamin E Determination in Tissue

Vitamin E was determined spectrophotometrically by a Hitachi spectrophotometer according to the method described by Desai (1984) with dl-α-tocopherol as a standard. The spectrophotometer was calibrated using quinine 0.1 mg/ml of 0.1 N sulfuric acid. In a control experiment dl-α-tocopherol was added to tissue homogenates and yield of the extraction procedure was measured. In most of the cases, yield of vitamin E extraction from the tissue was over 95%.

 Determination of Lipid-Soluble Fluorophore

The measurement of lipid-soluble fluorophore was performed as described previously (Dillard and Tappel 1984). Excitation and emission wavelengths were 360 nm and 460 nm, respectively. The concentration of fluorophore was expressed as relative fluorescence, where fluorescence of quinine 0.1 mg/ml of 0.1 N sulfuric acid is considered as 100. The concentration of protein was determined by the Lowry method, using bovine serum albumin as the standard (Lowry et al. 1951).

Statistical Analysis

All the data are presented as means ± SD. The statistical analysis of data was carried out by analysis of variance (ANOVA) with a Duncan post hoc test.

Results

Effect of Hydrazine Treatment on the Level of vitamin E

As shown in Table 1, rats treated with 0.5% hydrazine in the diet for 7 days demonstrated pronounced changes in vitamin E distribution. Liver, heart, spleen, and muscle tissues demonstrated a significant increase in vitamin E content after hydrazine treatment compared with control levels. The increases were 215% in liver, 118% in heart, 135% in spleen, and 100% in muscle over control values. However, no significant changes in vitamin E levels were observed in kidney and pancreas. On the other hand, concentration of vitamin E in the serum dropped to 51% of control level (Table 1).

Treatment of rats with vitamin E (700 mg/kg per body weight) during a 10-day period led to large accumulation in rat tissues. The highest accumulation of vitamin E was observed in pancreas and spleen. Comparing the average values of accumulated vitamin E in the studied tissues, the order of increase is as follows: pancreas > spleen > kidney > muscle > liver > heart. Moreover, the serum level of vitamin E was also elevated over the control value (Table 1).