Distribution and Metabolism of Two Orally Administered Esters of Tocopherol

HUGO E. GALLO-TORRES, O. NEAL MILLER, JAMES G. HAMILTON and CAROL TRATNYEK,
Department of Biochemical Nutrition, Hoffman-La Roche Inc., Nutley, New Jersey 07110

ABSTRACT

A comparison of the distribution of total radioactivity in rat tissue lipids after the oral administration of d,1-3,4-3H2-α-tocopheryl nicotinate and d,1-α-tocopheryl-1',2'-3H2-acetate in equimolar concentrations has demonstrated that there is considerable variation in the concentration of vitamin E in organs at different times after dosing. A higher total radioactivity was found in the tissues of animals receiving α-tocopheryl nicotinate than after α-tocopheryl acetate 12 hr after feeding with an emulsion, but not at most other time intervals studied. These findings indicate that the tissue uptake of vitamin E after oral dosage with nicotinate ester is, perhaps, poorer than that occurring after feeding with tocopheryl acetate, or that α-tocopheryl nicotinate has a faster turnover than the acetate ester. Although total radioactivity in the blood and liver of those animals dosed with α-tocopheryl acetate varied slightly with time, there was a high peak of radioactivity at 12 hr after dosage with nicotinate ester. In both groups of rats, the adrenals, ovaries, adipose tissue and heart appeared to extract vitamin E from the blood for a period of up to 48 hr postabsorptively. Metabolic products of tocopherol detected by glass-fiber paper chromatography were found in both instances. This analysis revealed that, when orally administered, both α-tocopheryl nicotinate and α-tocopheryl acetate are extensively metabolized by the tissues of the rat. The metabolite most abundantly occurring under these conditions was α-tocopheryl quinone. In the adrenal glands, however, the most highly labeled compound was unesterified tocopherol, which increased with time and comprised up to 90% of the chromatographed radioactivity. From the data obtained, it can be assumed that the adrenal tissue plays a definite role in the metabolism of vitamin E.

INTRODUCTION

Evidence has recently been presented (1,2) indicating that a higher concentration of tocopherol appears in the lymph when α-tocopheryl nicotinate is administered to rats with a thoracic duct fistula than when α-tocopheryl acetate is given. This difference in absorption and lymphatic transport could result in higher tissue concentrations of vitamin E following oral dosage with the nicotinate ester. Because it is possible that some of the suspected biological activities of vitamin E (such as its possible “anti-inflammatory action”) can take place only when the vitamin is present in adequately high concentrations at its site of action (target tissue), we have engaged in the search of vitamin E derivatives capable of producing high tissue concentrations of this vitamin.

The object of the present investigation was to determine the organ distribution, storage and metabolism of orally administered α-tocopheryl nicotinate, and to correlate these findings with those obtained after the administration of α-tocopheryl acetate. The latter was selected for comparison purposes because it constitutes the most widely used form of vitamin E in animals and humans.

EXPERIMENTAL PROCEDURES

Female albino rats (CD Charles River Breeding Labs., North Wilmington, Mass.), weighing between 230 and 250 g, were maintained on a diet of laboratory Purina chow and water, ad lib. The animals were fasted overnight before administration by stomach tubes of an emulsion containing labeled vitamin E ester. The emulsion (4 ml) was of the same composition as that utilized in studies of cholesterol absorption and has been described in detail elsewhere (3). Ethanolic solutions containing 2.3 mg of d,l-α-tocopheryl nicotinate and 50 μC of d,1-3,43H2-α-tocopheryl nicotinate mixed with 2.3 mg of d,1-3,43H2-α-tocopheryl nicotinate mixed with 2.3 mg of d,1-3,43H2-α-tocopheryl nicotinate were added to the emulsion and administered to a group of rats. Another group of animals received the same emulsion in which had been dissolven ethanolic solutions of 2 mg.

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2Present address: Department of Vitamin and Nutritional Research F. Hoffman-La Roche & Co., 4002 Basle, Switzerland.
of d,l-α-tocopheryl acetate and 50 μC of d,1-α-tocopheryl-1',2'-3H2-acetate. In both instances the test emulsion was given with the aid of a steel oral cannula to unanesthetized animals. The rats were subsequently confined to restraining cages to prevent coprophagy, and were allowed to drink .85% saline ad lib. during the entire duration of the experiment. Both the radioactive substances and the vitamin E carrier esters were products synthesized at Roche Laboratories. When checked for purity by either thin layer silicic acid or glass-fiber paper chromatography using liquid scintillation counting (1-3), the radioactive substances were found to be > 99% pure; these compounds were, therefore, used without further purification.

Collection of Samples

The animals were subdivided into groups of two, taken out of the restraining cages, anesthetized with Penthrane (Methoxyfluorane, Abbott Laboratory, North Chicago, Ill.) and killed 3, 6, 12, 24 or 48 hr after administration of the emulsion. As much blood as possible was taken from the abdominal aorta. The samples of blood were collected with heparinized tubes. A sample of skeletal muscle was taken from both thighs and adipose tissue was removed immediately after taking the blood sample, rinsed two or three times with distilled water, weighed and kept frozen until further processing.

Tissue Lipid Extraction and Radioactive Measurements

Samples of total blood were extracted and processed as described for cholesterol absorption studies (3). The extracting solvents were purified as reported previously (3). The heart, skeletal muscle, liver and kidney were homogenized with a minimum amount of water in a drill press (Sears, Roebuck and Co.) at 1000 rpm. After complete homogenization was achieved, the organs were lyophilized in a Virtis Freeze-Drying Unit and the lipids were subsequently extracted with varying amounts of ethanol-isopropyl ether (2:1).

The spleen, adrenals, ovaries and adipose tissue were homogenized vigorously and extracted simultaneously with ethanol-isopropyl ether, using 30 ml extracting solvent per g of wet tissue.

After extraction, the samples were centrifuged at 2000 rpm, the supernatant phase removed, evaporated to dryness under N2, and finally dissolved in 0.4 to 4 ml volumes of extracting mixture. To measure total radioactive activity, one aliquot was applied to a small piece of glass-fiber paper and allowed to dry. The radioactivity was then counted. Another aliquot was utilized to separate the tocopherol and its metabolites from the lipids. This was done by glass-fiber paper chromatography (ITLC-SG, Gelman Instruments Co., Ann Arbor, Mich.). Solvent systems recently developed for the separation of vitamin E metabolites in lymph (2) were used. In all instances, measurement of radioactivity was achieved by using a Packard Tri-Carb liquid scintillation Spectrometer, with Model 544 attachment (1). The results are thus reported in dpm/g wet tissue and there was no significant quenching by the samples used.

RESULTS AND DISCUSSION

Figure 1 illustrates the appearance of total radioactive vitamin E in the blood, liver, spleen and kidney of rats orally dosed with either α-tocopheryl nicotinate or α-tocopheryl acetate. The uptake of radioactivity resulting from administered nicotinate ester was higher than that from acetate ester feeding only 12 hr after administration of the emulsion. At most other experimental periods a higher uptake of vitamin E after dosage with α-tocopheryl acetate was observed than after α-tocopheryl nicotinate. After dosage with α-tocopheryl acetate the labeled vitamin E in the blood increased slightly with time. This concentration of radiovitamin E in the blood is the result of a balance between the rates of absorption from the gastrointestinal tract, release by the liver and the concomitant metabolism by peripheral tissues.

Both α-tocopheryl nicotinate and α-tocopheryl acetate were readily converted in the studied animals to free tocopherol and its metabolites (see Table I). This conversion presumably started at the level of the intestinal mucosa (1,2). After dosage with either of the two tocopherol esters, most of the radioactivity was recovered as tocopherol quinone. Under the present experimental conditions, there was a significantly higher concentration of free tocopherol in the blood after the administration of vitamin E acetate than after nicotinate, thus indicating that dosage with α-tocopheryl acetate is of greater effect than α-tocopheryl nicotinate as regards α-tocopherol in the blood. It is possible that these differences in concentration in the blood tocopherol of the two groups of animals may correlate with differences in the biological activity of these two tocopherol esters.

It was previously shown that independently of the nature of the administered tocopherol