Selective Uptake of Dietary Tocotrienols into Rat Skin

Saiko IKEDA,* Takako NIWA and Kanae YAMASHITA
Department of Food and Nutrition, School of Life Studies, Sugiyama Jogakuen University,
17-3 Hoshigaoka-motomachi, Chikusa-ku, Nagoya 464–8662, Japan
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Summary Using a vitamin E mixture extracted from palm oil, the tissue distribution of dietary tocotrienols and tocopherols was examined in rats and mice. Wistar rats (4-wk-old) were fed a diet containing 48.8 mg/kg α-tocopherol, 45.8 mg/kg α-tocotrienol and 71.4 mg/kg γ-tocotrienol for 8 wk. Nude mice (BALB/c Slc- nu, 8-wk-old) and hairless mice (SKH1, 8-wk-old) were fed the same diet for 4 wk. α-Tocopherol was abundantly retained in the skin, liver, kidney and plasma of rats and mice. α-Tocotrienol and γ-tocotrienol were detected slightly in the liver, kidney and plasma, while substantial amounts of these tocotrienols were detected in the skin of both rats and mice. The present study suggests that the skin is a unique tissue in respect to its ability to discriminate between various vitamin E analogs.

Key Words tocotrienol, vitamin E, rat skin, mouse skin

Vitamin E is a potent fat-soluble antioxidant and inhibits lipid peroxidation in biological membranes. In nature, compounds with vitamin E activity are α-, β-, γ- or δ-tocopherols and α-, β-, γ- or δ-tocotrienols. The chemical properties of these vitamin E analogs include antioxidative activities. The antioxidative activities of α-tocotrienol to lipid peroxidation in rat microsome and mitochondria, and the oxidation of dioleoylphosphatidylcholine liposomes, are known to be higher than that of α-tocopherol (1–3). But the antioxidative activities of vitamin E analogs in vitro are poorly correlated to their biological activities, and α-tocopherol has the highest biological activity among the vitamin E analogs. α-Tocopherol is a major form of vitamin E in tissues and plasma, in spite of there being a great deal of dietary γ-tocopherol. This discrimination between α-tocopherol and other analogs is accomplished by the action of αTTP expressed in the liver (4).

αTTP was reported by Sato et al. to have the capacity to bind α-tocopherol in the rat liver (5). Dietary vitamin E analogs are absorbed at the intestine and carried to the liver as a result of the uptake of chylomicron remnants (6). There is no discrimination between α-tocopherol and other analogs during absorption and chylomicron secretion by the intestine (7, 8). αTTP catalyzes α-tocopherol secretion by a novel non-Golgi-mediated pathway in liver cells, and α-tocopherol is incorporated into VLDL and transported to the various tissues by lipoproteins (4). The other analogs of vitamin E such as tocotrienols and γ-tocopherol are excreted because their affinity for αTTP is low. Hosomi et al. reported that the relative affinity of tocopherol analogs or α-tocopherol for αTTP correlated well with their biological activity (9). In addition, a patient who had ataxia with vitamin E deficiency also had an extremely low vitamin E concentration in the plasma, which was caused by mutations in the αTTP gene (10). Thus, the affinity of vitamin E analogs for αTTP is a critical determinant of their biological activity, and the low biological activities of tocotrienols may account for their low affinity for αTTP. We previously reported that α-tocotrienol concentrations in the liver, kidney, testis, adrenal gland and plasma were extremely low in the rats fed the diet containing α-tocotrienol (11).

However, Podda et al. recently reported that the tocotrienol concentration in the skin was higher than that in the brain, heart, kidney or liver in hairless mice fed a commercial diet containing a small amount of tocotrienols (12). Skin is the tissue most directly exposed to ultraviolet rays and oxygen. Exposure to them may cause the oxidation and peroxidation of various molecules in the skin. Traber et al. reported that topically applied α- and γ-tocotrienols penetrated into the skin of hairless mice (13). Moreover, Thiele et al. reported that the topical application of α- and γ-tocotrienols from an α-tocotrienol-rich fraction of palm oil prevented the lipid peroxidation caused by ozone exposure (14). If dietary tocotrienols can be taken up by the tissue, the tocotrienols would take on the function of vitamin E. Because the tocotrienol concentration in the rat skin has not been examined, we examined the tissue distribution of dietary tocotrienols and tocopherols in rats using a vitamin E mixture extracted from palm oil. We also examined the distribution in hairless mice and nude mice in order to compare rats with mice.

Experimental

Materials. Vitamin E mixture extracted from palm oil and α-tocopherol, γ-tocopherol, α-tocotrienol and
γ-tocotrienol for use as standards were kindly provided by Lion (Tokyo, Japan). The vitamin E mixture consisted of 24.5% α-tocopherol, 0.8% β-tocopherol, 1.2% γ-tocopherol, 0.6% δ-tocopherol, 22.8% α-tocotrienol, 2.9% β-tocotrienol, 38.4% γ-tocotrienol and 8.8% δ-tocotrienol.

Animals and diets. Male Wistar rats 4 wk of age and female nude mice (BALB/c SLC-nu) 8 wk of age were purchased from Japan SLC (Shizuoka, Japan). Female hairless mice (SKH1) 8 wk of age were purchased from Charles River Laboratories (Yokohama, Japan). Both rats and mice were maintained at 24.5°C with a 12-h light cycle (lights on from 0800 to 2000) and allowed free access to water and a purified diet. The composition of the diet (g/kg) was as follows: casein (vitamin free), 200; sucrose, 100; starch, 502.27; cellulose powder, 50; AIN93 mineral mixture (15), 35; AIN93 vitamin mixture (vitamin E free) (15), 10; choline bitartrate, 2.5; corn oil (vitamin E free), 100; vitamin E mixture, 0.22; and butylated hydroxytoluene, 0.01. The calculated contents of vitamin E analogs in this diet (mg/kg) were as follows: α-tocopherol, 50; β-tocopherol, 1.5; γ-tocopherol, 2.4; δ-tocopherol, 1.3; α-tocotrienol, 46.5; β-tocotrienol, 5.9; γ-tocotrienol, 78.4; and δ-tocotrienol, 17.8. Rats were fed this diet for 8 wk and mice for 4 wk. After fasting for 24 h, animals were anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories), and blood was drawn from the heart using heparinized needles and syringes. Dorsal skin, liver and kidney were taken and stored at -80°C until use for determination of tocopherol and tocotrienol concentrations. All procedures were performed in accordance with the Animal Experimentation Guides of Nagoya University.

Tocopherol and tocotrienol concentrations. Tocopherols and tocotrienols were extracted from the plasma, liver and kidney as described previously (16). Skin was handled using the method of Podda et al. (12). Briefly, the frozen skin was weighed, ground under liquid nitrogen, and homogenized in 10 mM sodium phosphate buffer containing 130 mM NaCl, 1 mM EDTA, and 1.13 mM butylated hydroxytoluene (pH 7.0). The homogenate was supplemented with 1 mL of 0.1 M SDS and mixed for 30 s. After the addition of 2 mL ethanol, the homogenate was extracted with 2 mL of hexane and an appropriate aliquot was dried and suspended in hexane. Tocopherol and tocotrienol concentrations were determined by the HPLC method (17).

Results and Discussion

The vitamin E content in the diet determined by HPLC is shown in Table 1. The content of α-tocopherol, α-tocotrienol and γ-tocotrienol in the diet was 97, 98 and 91% of the calculated value, respectively. The difference between the actual content and the calculated value may be due to the experimental error when the components of the purified diet were mixed. The vitamin E content in the skin, liver, kidney and plasma of rats, nude mice and hairless mice are shown in Fig. 1. In the rats, the vitamin E detected in the liver, kidney and plasma was exclusively α-tocopherol, in spite of there being many tocotrienols in the diet. In contrast, the skin contained α-tocopherol, α-tocotrienol and γ-tocotrienol, although the α-tocopherol concentration in the skin was lower than that in the liver and kidney. The most abundant vitamin E analog in the diet was γ-tocotrienol (Table 1), but the skin concentration of α-tocotrienol was higher than that of γ-tocotrienol. The methyl group at position 5 on the chromanol ring of tocotrienol may be important for the discrimination in the skin. In the skin of the nude mice and hairless mice, α-tocopherol, and α- and γ-tocotrienols were contained, and the α-tocotrienol concentration was higher than that of γ-tocotrienol. Only α-tocopherol was contained in the liver and plasma. Tocotrienols were detected in the kidney of the nude mice but their concentrations were extremely low. These data indicate that dietary tocotrienols, which have low affinity for αTTP, were taken up into the skin, but not into the liver and kidney in rats and mice. This suggests the existence of

<table>
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<tr>
<th>Vitamin E analog</th>
<th>Amount (mg/kg diet)</th>
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<tr>
<td>α-Tocopherol</td>
<td>48.8±0.7</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>not detected</td>
</tr>
<tr>
<td>α-Tocotrienol</td>
<td>45.8±0.4</td>
</tr>
<tr>
<td>γ-Tocotrienol</td>
<td>71.4±0.5</td>
</tr>
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Values are means±SE.

Fig. 1. Concentrations of α-tocopherol, γ-tocopherol, α-tocotrienol and γ-tocotrienol in the skin, liver, kidney and plasma of rats, nude mice and hairless mice. Values are means±SE, n=6. Rats were fed the diet for 8 wk, and both nude mice and hairless mice were fed the diet for 4 wk. The animals were killed after fasting for 24 h.
another pathway transporting vitamin E analogs to the skin, which is independent of their affinity for αTTP.

Since αTTP was identified and characterized, it has been thought that the discrimination between α-tocopherol and other analogs is mainly due to the action of αTTP. Dietary vitamin E is absorbed in the intestine and carried to the liver as a result of the uptake of chylomicron remnants. In rats, the lymphatic transport of α-, γ- and δ-tocotrienols was not repressed in comparison with that of α-tocopherol (8). α-Tocopherol with VLDL is secreted into the blood and transported to the various tissues. Other vitamin E analogs such as tocotrienols and γ-tocopherol are excreted because of their low affinity for αTTP. In this study, few chylomicrons and chylomicron remnants were contained in the plasma because the plasma was taken from the animals after 24-h fasting. The fact that tocotrienols could not be detected in the liver and plasma agree with the idea of a discrimination system by αTTP. Tocotrienols contained in the skin suggest the existence of another pathway that was independent of αTTP because the affinity of α-tocotrienol for αTTP was extremely low (9). It has been reported that LPL (E.C. 3.3.1.34) has the ability to transfer vitamin E (6, 7). LPL is synthesized in various peripheral tissues (19). It was reported that LPL (E.C. 3.3.1.34) has the ability to transfer vitamin E (6, 7). LPL is synthesized in various tissues, such as the adipose tissue, heart and skeletal muscle and the mammary gland, and acts at the surface of endothelial cells in the capillaries (18). LPL catalyzes the hydrolysis of triglycerides in plasma lipoprotein and makes the fatty acids available for use by peripheral tissues (19). It was reported that γ-tocopherol was transferred to the human fibroblasts during hydrolysis of triglycerides by bovine LPL (20). Therefore, tocotrienols contained in the skin in this study may be transferred by LPL from chylomicron directly, not through the liver.

Some reports suggest that α-tocotrienol is a more effective antioxidant in vitro (1–3). Therefore, it is interesting that dietary tocotrienol was taken up to the skin of rats in this study. More studies are needed for understanding the biokinetics of tocotrienols and their physiological activities.

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REFERENCES