Comparative Changes in Plasma and RBC α -Tocopherol after Administration of *dl*- α -Tocopheryl Acetate and *d*- α -Tocopherol

Toru OGIHARA, Yasuhiko NISHIDA, Masayuki MIKI,
and Makoto MINO¹

*Department of Pediatrics, Osaka Medical College,
Takatsuki 569, Japan*

(Received October 5, 1984)

Summary Changes in plasma and RBC α -tocopherol after intravenous and oral administration of *dl*- α -tocopheryl acetate were investigated using vitamin E-deficient rats and were compared with those following the administration of *d*- α -tocopherol, which is the most active and natural form of tocopherol.

1. Intravenous administration: By the administration of *dl*-tocopheryl acetate, the elevated plasma tocopherol levels were higher at 6 h than those elevated by *d*-tocopherol, but the RBC tocopherol was lower 6 h after the infusion of *dl*-tocopheryl acetate than after *d*-tocopherol infusion. The tocopheryl acetate incorporated into the RBCs remained for 24 h after the administration of tocopheryl acetate, the acetate then becoming undetectable in plasma.

2. Oral administration: The tocopherol elevated in plasma and RBC was only the α -form and not tocopheryl acetate. In both plasma and RBCs, tocopherol levels were higher after the administration of *d*-tocopherol than after *dl*-tocopheryl acetate administration, and more was found in RBCs.

Key Words vitamin E, tocopheryl acetate, plasma, RBC, hydrolysis

There has recently been renewed interest in the clinical use of large amounts of vitamin E to reduce the serious ocular sequelae of retrolental fibroplasia (1–4) and the neurological sequelae of lipid malabsorption (5–7). Mega-doses used in vitamin E therapy have increased in order to maintain high plasma vitamin E, which is thought to act as an antioxidant localized in biological membranes. Thus, the ultimate purpose of this therapy is to maintain higher tocopherol levels in biomembranes but not in plasma. The therapeutic compounds of vitamin E usually used in Japan are tocopheryl esters, tocopheryl acetate or nicotinate. In this paper,

¹ 荻原 享, 西田靖彦, 三木正之, 美濃 真

we have investigated the changes in α -tocopherol in plasma and RBCs after the administration of *dl*- α -tocopheryl acetate, which were compared with those following the administration of *d*- α -tocopherol as the most active and natural form of tocopherols. RBC tocopherol was used as the index of physiologically available tocopherol localized in biomembranes (8).

MATERIALS AND METHODS

Animals. The animals used were Wistar-strain rats, 3 weeks of age and each weighing about 50 g. The start of feeding with a vitamin E-deficient basal diet was preceded by 2 weeks' feeding with a control diet prepared by the addition of 2 mg of *dl*- α -tocopheryl acetate to 100 g of the basal diet. The vitamin E-deficient diet was composed of 36% corn starch, 25% vitamin-free casein, 10% alpha wheat starch, 8% powdered filter paper, 6% salt mixture, 5% granulated sugar, 2% vitamin mixture, and 8% stripped corn oil (from Eastman Kodak Chemicals); the salt mixture: K 692 mg, P 579 mg, Ca 411 mg, Na 270 mg, Mg 86 mg, Fe 41 mg, Zn 0.4 mg, Mn 1.3 mg, and I 7.7 mg per 100 g diet; the vitamin mixture: vitamin A 1,000 IU, D₂ 200 IU, B₁ 2.4 mg, B₂ 8 mg, B₆ 1.6 mg, B₁₂ 0.001 mg, C 60 mg, K 10.4 mg, biotin 0.04 mg, folic acid 0.4 mg, Ca-pantothenate 10 mg, *p*-aminobenzoic acid 10 mg, niacin 12 mg, inositol 12 mg, and choline-Cl 4,000 mg per 100 g diet. Vitamin E deficiency in rats occurred after more than 8 weeks' feeding of the basal vitamin E-deficient diet.

Administration of tocopherol to the vitamin E-deficient rats. The water-dispersible preparations of *d*- α -tocopherol (4 mg/ml) and *dl*- α -tocopheryl acetate (4 mg/ml, tocopherol equivalent) for intravenous use were supplied by Nihonkayaku Co., Ltd. After the vitamin E-deficient rats had fasted for 24 h, respective preparations were given at a dose of 10 mg/kg either intravenously or orally. Oral administration of the preparations for intravenous use was carried out using a vinyl catheter.

Preparation of blood for tocopherol assay. Heparinized blood was centrifuged at 3,000 rpm for 10 min to separate RBC from plasma. The RBCs were washed three times, each time with 10 volumes of *n*-saline. At the last washing, centrifugation was maintained at exactly 3,000 rpm for 10 min to obtain a constant packed cell volume.

Tocopherol assay. The assay method used was a slight modification of Katsui's method (9), as follows. The samples (0.5 ml) were placed in a centrifuge tube together with 1 ml 6% pyrogallol solution in ethanol, and with synthetic *dl*-tocol solution (2 μ g/ml ethanol) as an internal standard. 1.5 ml of 0.15 M phosphate buffer, pH 7.4, and 5 ml hexane were added to the tube, which was vigorously shaken for 5 min. The mixture was centrifuged at 3,000 rpm for 5 min, and 4 ml of the hexane layer was removed and evaporated under nitrogen gas flow at 40°C. The residue was dissolved in 100 μ l isopropyl alcohol and 20 μ l was injected into the HPLC column.

The instruments used in this study were as follows. HPLC, Shimadzu LC-5A

Fig. 1. Chromatographic pattern of tocol, α -tocopherol, and *dl*- α -tocopheryl acetate analyzed by HPLC. Retention time was 6.35 min for tocol, 7.45 min for tocopherol, and 8.9 min for tocopheryl acetate.

(Shimadzu Co., Ltd., Kyoto), Column, SSC-ODS (Sensyu Kagaku Co., Ltd., Tokyo) (4.6 i.d. $\times$ 200 mm). Eluent, ethanol: methanol (90: 10). Flow rate 0.5 ml/min. Detector, Shimadzu SPD-2A (UV spectrometer) at 290 nm. The elution patterns of tocopherol, tocopheryl acetate and tocol separated by HPLC are shown in Fig. 1. The RBC tocopherol levels obtained from HPLC analysis were corrected by hematocrit values in each sample.

RESULTS AND DISCUSSION

1. Intravenous administration

Figure 2 shows the changes in plasma tocopherol levels after intravenous administration of *dl*- α -tocopheryl acetate (10 mg/kg, tocopherol equivalent). After 6 h, the total plasma tocopherol level was more than 7,000 μ g/100 ml which involved about 64% of tocopheryl acetate. Therefore, 36% of the tocopheryl acetate infused was apparently hydrolyzed in the body. After more than 24 h, no detectable tocopheryl acetate was observed in the plasma. Newmark *et al.* (10) have made studies using dogs and reported that only one-fourth of the intravenously infused tocopheryl acetate was apparently hydrolyzed after 24 h, on the basis of the findings of plasma tocopheryl acetate and tocopherol levels. The difference between the

Fig. 2. Tocopherol and tocopheryl acetate in plasma after intravenous administration of 10 mg/kg of *dl*- α -tocopheryl acetate in vitamin E-deficient rats (percentage shows the remaining acetate). Each bar represents the mean of 5 rats.

Fig. 3. Tocopherol and tocopheryl acetate in RBC after intravenous administration of 10 mg/kg of tocopheryl acetate in vitamin E-deficient rats (percentage shows the remaining acetate). Each bar represents the mean of 5 rats.

reports of Newmark and ourselves as regards hydrolytic capability, possibly stems from the difference in animal species. In RBCs, a marked elevation of tocopherol levels was also observed at 6 h, tocopheryl acetate being found in 66% of the elevated total RBC tocopherol. The total tocopherol decreased in RBCs at 24 h while 20% of the tocopheryl ester remained in the total tocopherol, as shown in Fig. 3. This indicates that a part of the injected tocopheryl acetate was incorporated into the RBC membrane unless it had been previously hydrolyzed and remained longer in the membranes. Figures 4 and 5 show the changes in α -tocopherol alone in plasma and RBCs, respectively. The elevated tocopherol was higher in plasma at 6 h after the administration of *dl*- α -tocopheryl acetate than after *d*- α -tocopherol administration ($p < 0.02$). In contrast to the plasma changes, RBC tocopherol was much

Fig. 4. Changes in plasma tocopherol after intravenous administration of 10 mg/kg *dl*- α -tocopheryl acetate and *d*- α -tocopherol, respectively, in vitamin E-deficient rats. Each point represents the mean $\pm$ SD ($n=5$). Level of significance of difference: ** $p < 0.02$, ns (not significant).

Fig. 5. Changes in RBC tocopherol after intravenous administration of 10 mg/kg *dl*- α -tocopheryl acetate and *d*- α -tocopherol, respectively, in vitamin E-deficient rats. Each point represents the mean $\pm$ SD ($n=5$). Level of significance of difference: ** $p < 0.01$, ns (not significant).

higher at 6 h after the administration of *d*- α -tocopherol than after *dl*- α -tocopheryl acetate administration ($p < 0.01$). The discrepancy between plasma and RBC may be explained as follows. The *d*- α -tocopherol is directly incorporated into the biological membranes, including RBC membranes, during circulation, while *dl*- α -tocopheryl acetate is first hydrolyzed by tissue esterase, and the tocopherol produced by hydrolysis is taken up by the membranes, together with the unhydrolyzed acetate. Therefore, the higher concentration of RBC tocopherol accompanied by the lower one of plasma tocopherol at 6 h after *d*- α -tocopherol administration, may be attributable to the faster incorporation into biomembranes. The finding of the lower value for RBC tocopherol and the higher one for plasma tocopherol at 6 h after the infusion of *dl*- α -tocopheryl acetate may be related to the time-lag for hydrolysis of the acetate administered. Although the difference in tocopherol levels between plasma and RBCs at 6 h after the administration disappeared at 24 h and 48 h ($p > 0.05$), it may be undesirable that tocopheryl acetate remains in RBC membranes for more than 24 h.

2. Oral administration

Figures 6 and 7 show the changes of tocopherol in plasma and RBCs after oral administration. When given with *dl*- α -tocopheryl acetate, α -tocopherol alone was detected in plasma and RBCs. This indicates that tocopheryl acetate is completely hydrolyzed in the intestine during absorption. In both plasma and RBCs, tocopherol levels were higher after the administration of *d*- α -tocopherol than after that of *dl*- α -tocopheryl acetate ($p < 0.01$ at 6 and 24 h in plasma), and more was found in RBCs ($p < 0.001$ at all the points analyzed after the administrations). This shows that free tocopherol is more effectively absorbed through the gut and more easily

Fig. 6. Changes in plasma tocopherol after oral administration of 10 mg/kg *dl*- α -tocopheryl acetate and *d*- α -tocopherol, respectively, in vitamin E-deficient rats. Each point represents the mean $\pm$ SD ($n = 5$). Level of significance of difference: ** $p < 0.001$, * $p < 0.01$, ns (not significant).

Fig. 7. Changes in RBC tocopherol after oral administration of 10 mg/kg *dl*-α-tocopheryl acetate and *d*-α-tocopherol, respectively, in vitamin E-deficient rats. Each point represents the mean $\pm$ SD ($n=5$). Level of significance of difference: ** $p < 0.001$.

incorporated into the membranes than the tocopheryl ester. Horwitt (11) compared the relative potencies of *d*-α-tocopheryl acetate to those of *dl*-α-tocopheryl acetate when used as a supplement for vitamin E-depleted human adult subjects, and reported that *dl*-α-tocopheryl acetate may have no more than half the biological potency of *d*-α-tocopheryl acetate on the basis of the finding of elevating plasma tocopherol levels after oral administration. More recently, Horwitt *et al.* (12) studied again the serum concentration of α-tocopherol after ingestion of *d*-α-tocopheryl acetate, *dl*-α-tocopheryl acetate, *d*-α-tocopherol, *d*-α-tocopheryl succinate, and *d*-α-tocopheryl acetate plus apple pectin by each of 20 adult human subjects, and reported that the mean increase in concentration of α-tocopherol in serum was the largest during the 24 h following oral ingestion of *d*-α-tocopherol, while it was the smallest after oral ingestion of *dl*-α-tocopheryl acetate and *d*-α-tocopheryl succinate. In the view of Horwitt's data on serum tocopherol changes, *d*-α-tocopherol seems to be more potent than *d*-α-tocopheryl acetate which in turn may be more potent than *dl*-α-tocopheryl acetate. The serum changes in tocopherol levels between *d*-α-tocopherol and *dl*-α-tocopheryl acetate agreed with our finding using rats. Other previous data of Week *et al.* (13), Baker *et al.* (14), and Hung *et al.* (15) support Horwitt's view. In particular, Hung *et al.* (15) have reported that the concentrations of tocopherols in plasma, liver, kidney, spleen, and heart of rainbow trout increase six to 18 times more in the first 4 h after oral administration of *d*-α-tocopherol than did *dl*-α-tocopheryl acetate. Although there is no guarantee that the relative activities of the tocopherols established by the trout experiment are the same in mammals, the changes in tocopherol concentrations in the trout tissues observed by Hung *et al.* were consistent with those in RBCs of our study. In our study, special attention was paid to the relative potencies of *dl*-α-tocopheryl acetate

compared with *d*- α -tocopherol which is thought to be natural and most active, since *dl*- α -tocopheryl acetate is used in most clinical supplementations. Our finding that RBC tocopherol was usually at higher levels after administration of *d*- α -tocopherol than after that of the *dl*- α -tocopheryl acetate either intravenously or orally, may provide an evaluation of the reliability of the clinical use of *d*- α -tocopherol, because of RBC tocopherol being used as the index of physiologically available tocopherol localized in biomembranes (8). Therefore, it would be helpful to provide a preparation of α -tocopherol, but not tocopheryl esters, for clinical use.

The authors are grateful to Mr. S. Nakagawa, B. S., the Godai Embody Co., Ltd. for his helpful advice, and to the Eisai Co., Ltd. for providing the vitamin E-deficient diets and tocol as an internal standard. The authors also thank Nihonkayaku Co., Ltd. for the gift of intravenous preparations of *d*- α -tocopherol and *dl*- α -tocopheryl acetate.

This investigation was supported by a Grant-in-Aid (58480260) for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

- 1) Johnson, L., Schaffer, D., Quinn, G., Goldstein, D., Mathis, M. J., Otis, C., and Boggs, T. R., Jr. (1982): Vitamin E supplementation and the retinopathy of prematurity. *Ann. N. Y. Acad. Sci.*, **393**, 473–495.
- 2) Finner, N. N., Schindler, R. F., Grant, G. D., and Hill, G. B. (1982): Effect of intramuscular vitamin E on frequency and severity of retrolental fibroplasia. A controlled trial. *Lancet*, **1**, 1087–1092.
- 3) Finner, N. N., Schindler, R. F., Peter, K. L., and Grant, G. D. (1983): Vitamin E and retrolental fibroplasia: Improved visual outcome with early vitamin E. *Ophthalmology*, **90**, 428–435.
- 4) Hittner, H. M., Speer, M. E., Rugolph, A. J., Blifeld, C., Chadda, P., Holbein, B., Godio, L. B., and Kretzer, F. L. (1984): Retrolental fibroplasia and vitamin E in the preterm infant—Comparison of oral *versus* intramuscular: oral administration. *Pediatrics*, **73**, 238–249.
- 5) Elias, E., Muller, D. P. R., and Scott, J. (1982): Association of spinocellebellar disorders with cystic fibrosis or chronic childhood cholestasis and very low serum vitamin E. *Lancet*, **2**, 1319–1321.
- 6) Bishara, S., Merin, S., Cooper, M., Azizi, E., Delpre, G., and Deckelbaum, R. J. (1982): Combined vitamin A and E therapy prevents retinal electrophysiological deterioration in abetalipoproteinemia. *Br. J. Ophthalmol.*, **66**, 767–770.
- 7) Guggenheim, M. A., Ringer, S. P., Silverman, A., and Grabert, B. E. (1982): Progressive neuromuscular disease in children with chronic cholestasis and vitamin E deficiency: Diagnosis and treatment with α tocopherol. *J. Pediatr.*, **100**, 51–58.
- 8) Mino, M., Nakagawa, S., Tamai, H., and Miki, M. (1982): Clinical evaluation of red blood cell tocopherol. *Ann. N. Y. Acad. Sci.*, **393**, 175–178.
- 9) Katsui, G. (1981): Assay methods of vitamin E: 4. High performance liquid chromatographic method. *Vitamins* (in Japanese), **55**, 267–273.
- 10) Newmark, H. L., Pool, W., Bauernfeind, J. C., and DeRitter, E. (1975): Biopharmaceutic factors in parenteral administration of vitamin E. *J. Pharmaceut. Sci.*, **64**, 655–657.
- 11) Horwitt, M. K. (1980): Relative biological values of *d*- α -tocopheryl acetate and all-rac-

- α -tocopheryl acetate in man. *Am. J. Clin. Nutr.*, **33**, 1856–1860.
- 12) Horwitt, M. K., Elliott, W. H., Kanjanangulpan, P., and Fitch, C. D. (1984): Serum concentrations of α -tocopherol after ingestion of various vitamin E preparations. *Am. J. Clin. Nutr.*, **40**, 240–245.
 - 13) Week, E. F., Sevigne, F. J., and Ellis, M. E. (1952): The relative utilization of α -tocopherol and α -tocopheryl acetate by humans. *J. Nutr.*, **46**, 353–359.
 - 14) Baker, H., Frank, O., DeAngelis, B., and Feingold, S. (1980): Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. *Nutr. Rep. Int.*, **21**, 531–536.
 - 15) Hung, S. S. O., Moon, T. W., Hilton, J. W., and Slinger, S. J. (1982): Uptake, transport and distribution of *dl*- α -tocopheryl acetate compared to *d*- α -tocopherol in rainbow trout (*Salmo gairdneri*). *J. Nutr.*, **112**, 1590–1599.