Enhancement of Oral Bioavailability of *d*-α-Tocopherol Acetate by Lecithin-Dispersed Aqueous Preparation Containing Medium-Chain Triglycerides in Rats

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In order to evaluate oral dosage forms of *d*-α-tocopherol acetate (VEA), *d*-α-tocopherol (VE) concentration in the plasma was examined following oral administration of three VEA preparations; lecithin-dispersed aqueous preparation, polysorbate 80 (PS-80)-solubilized aqueous solution and soybean oil solution. The lecithin-dispersed preparation gave the highest C_{max} and the largest AUC_{0-24h}, while T_{max} was delayed. In the thoracic duct fistula rat, no increase in VE plasma concentration was observed after intraduodenal administration of lecithin-dispersed VEA preparation, while VE appeared in the thoracic lymph, indicating that VE is absorbed from the lecithin-dispersed preparation *via* the lymphatic route. The delayed T_{max} and prolonged VE plasma concentration obtained with the lecithin-dispersed preparation in comparison with PS-80-solubilized aqueous solution could be explained by the different route of absorption.

Keywords *d*-α-tocopherol acetate; *d*-α-tocopherol; lecithin-dispersed preparation; lymphatic absorption; oral dosage form

d-α-Tocopherol acetate (VEA) is orally administered for prevention and therapy of vitamin E deficiency, and the dosage form is usually an oily solution. Since VEA has a very low solubility in water, some consideration is needed for developing orally available dosage forms. Recently, Tokumura *et al.* reported that the bioavailability of *d*-α-tocopherol (VE) from capsules filled with oily solution is higher than that from a solid dosage from.[1] On the other hand, lipid dispersion systems, such as emulsions and liposomes, are potentially useful dosage forms for lipid-soluble drugs. Furthermore, medium-chain triglycerides (MCTG) were shown to increase VE absorption.[2] With these points as background, we decided to investigate the usefulness of a lecithin-dispersed VEA aqueous preparation, a combination of lecithin liposomes with MCTG, as an oral dosage form. Thus, in this study, we examined the availability of VEA in rats by administering a lecithin-dispersed aqueous preparation containing MCTG in comparison with soybean-oil solution and polysorbate 80 (PS-80)-solubilized aqueous solution.

Experimental

Materials VEA, VE, lecithin, MCTG and *dl*-tocol were supplied by Eisai Co., Ltd. Lecithin was soybean phospholipids consisting of phosphatidylcholine (47—56%), phosphatidylethanolamine (20—24%), phosphatidylinositol (8—9%), phosphatidic acid (2—6%), lysophosphatidylcholine (3—4%), lysophosphatidylethanolamine (2%) and a little neutral lipids. The fatty acid composition of MCTG was 75% caprylic acid and 25% capric acid. Other reagents were of reagent grade.

TABLE I. Formulation of *d*-α-Tocopherol Acetate (VEA) Preparations

Composition[a)]	Preparations		
	Lecithin-dispersed	PS-80-solubilized	Soybean-oil solution
VEA (mg)	5	5	5
MCTG (mg)	1	1	0
Lecithin (mg)	5	0	0
PS-80 (mg)	0	5	0
Glycerol (mg)	33	33	0
Reagents for pH-adjustment (μg)	167.5	167.5	0
Distilled water	*ad lib.*	*ad lib.*	0
Soybean oil	0	0	*ad lib.*

a) Expressed as the amounts in 1 ml of each preparation.

VEA Preparations The formulations of three VEA preparations are listed in Table I. The compositions in the table are expressed as amounts of reagents in 1 ml of each preparation, but the preparation was carried out on a large scale. The lecithin-dispersed aqueous preparation was prepared as follows. The mixture of VEA, lecithin, MCTG and glycerol was sonicated with an ultrasonic disruptor, model UR-200P (Tomy Seiko Co., Ltd., Tokyo), at the maximum output for 8 min on ice. Then aqueous buffer solution was added to the sonicated mixture, followed by stirring. The resultant lecithin-dispersed preparation showed no lamellae of lipid bilayers around dispersed particles on transmission electron microscopic observation, suggesting that the preparation is not a liposome dispersion but an emulsion. The mean particle size was 91.0 nm, as determined by a Coulter counter (model N4, Coulter Electronics Inc., Florida).

Absorption Experiments Male Wistar rats weighing 170—250 g were used. Rats were fasted for 12 h prior to the administration of VEA preparations, but allowed water *ad libitum*. One of the preparations was administered at the VEA dose of 10 mg/kg by gastric intubation at 8:00 a.m. under light ethylether anesthesia and the rat was fixed in a restraining cage. Blood samples were taken from the tail artery at 0, 3, 6, 9, 12, 15 and 24 h following the administration.

In order to examine the lymphatic absorption, cannulation into the thoracic duct was carried out under urethane anesthesia based on the method of Bollman *et al.*[3] A VEA preparation was administered intraduodenally and blood samples were taken as described above. The thoracic lymph was also collected at 1, 2, 3, 6, 9, 12, 15 and 24 h following the administration. The animal was fixed on a platform throughout the experiments.

Plasma and lymph samples were stored at −20 °C until the analysis. Since the conversion of VEA to VE before reaching the circulation was confirmed, only VE concentrations in the plasma and the lymph were determined.

Analytical Method VE concentration in the plasma or the lymph was determined by high-pressure liquid chromatography. To 100 μl of plasma or lymph, 100 μl of distilled water and 300 μl of ethanol were added and the mixture was extracted with 2 ml of *n*-hexane containing *dl*-tocol as an internal standard. The organic layer was evaporated to dryness and the residue was re-dissolved in *n*-hexane. An aliquot of the *n*-hexane solution was injected into a high-pressure liquid chromatograph after filtration through a 0.5 μm pore size Teflon membrane (Nihon Millipore Kogyo, Yonezawa). An LC-5A high-pressure liquid chromatograph (Shimadzu, Kyoto) equipped with an RF-535 spectrofluorophotometer (Shimadzu; excitation wavelength, 290 nm; emission wavelength, 325 nm) was used in normal phase with a Polygosil-60 column (250 × 4.0 mm i.d., Chemco Scientific Co., Osaka). The mobile phase of *n*-hexane–isopropanol for the

assay of VE was 300 : 1.7 (by volume) and the flow rate was maintained at 1.5 ml/min. VE concentration was calculated from the ratio of the peak area to that of the internal standard, using the calibration curve.

Pharmacokinetic Analysis *AUC* was calculated by the trapezoidal method and the mean residence time (*MRT*) was calculated by model-independent statistical moment analysis.[4] The results were expressed as the mean ± standard error (S.E.). The statistical analysis was carried out by using Student's *t* test.

Results and Discussion

VEA is an oxidation-stable ester of VE and is orally administered, usually as an oil solution, to improve VE deficiency accompanying multiple biochemical, hematological and histological abnormalities. In order to investigate the effect of dosage forms on the bioavailability of VEA, VE concentrations in the plasma were examined following the administration of soybean oil solution, PS-80-solubilized aqueous solution or lecithin-dispersed aqueous preparation of VEA. No unchanged VEA was detected in the circulation following the administration of the three preparations. This is consistent with the findings of Gallo-Torres[5]; VEA intragastrically administered was hydrolyzed by bile and pancreatic juice and most of the vitamin appeared in the thoracic lymph as non-esterified VE. As shown in Fig. 1, VE concentration in the plasma of fasted rats was about 4 μg/ml and remained at this level all day when none of the preparations was administered. From this result, we may consider that the concentration of endogenous VE in an individual rat is equal to its plasma concentration just before the administration of VEA preparation. Thus, the increase in VE concentration after oral administration of VEA preparations was estimated by subtracting VE concentration just before the administration from each determined value. As is evident from Fig. 2, VE concentrations in the plasma increased following the administration of the three preparations, but the shape of the plasma concentration–time curve was markedly dependent on the preparation. The absorption of VE from PS-80-solubilized solution was the fastest of the three; T_{max} was about 6 h and the plasma concentration declined thereafter with the half-life of 8.2 h. The concentration at 24 h was not significantly different from that of the non-administered group. When VEA was administered as the solution of soybean oil, VE concentration in the plasma was unchanged during the initial 3 h and subsequently increased. C_{max} was maintained from 6 to 12 h and then the concentration decreased to the level of the non-administered group at 24 h. On the other hand, the lecithin-dispersed VEA preparation gradually increased VE concentration in the plasma; C_{max} (reached at 12 h) was the highest of the three preparations and thus the concentration at 24 h was significantly higher than that of the non-administered group.

Table II shows AUC_{0-24h} and *MRT* together with C_{max} and T_{max} values for the increase of VE following administration of the three VEA preparations. The largest AUC_{0-24h} was obtained with the lecithin-dispersed preparation, indicating an improvement of the bioavailability. On the other hand, *MRT* for the lecithin-dispersed preparation was also the largest of the three. The effect of these preparations on the fate of VE in the circulation or in the

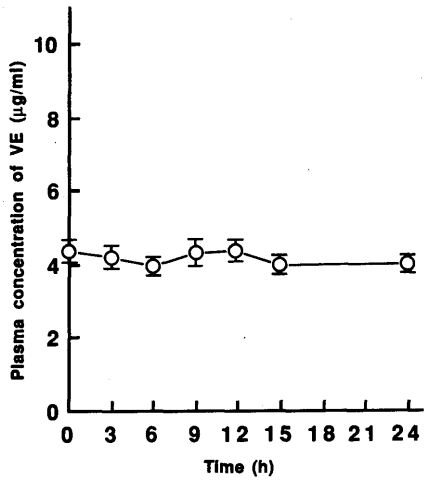

Fig. 1. Plasma Concentration of Endogenous *d*-α-Tocopherol (VE) in Fasted Rats

The blood sampling was started at 8:00 a.m. Results are expressed as the mean ± S.E. of 6 rats.

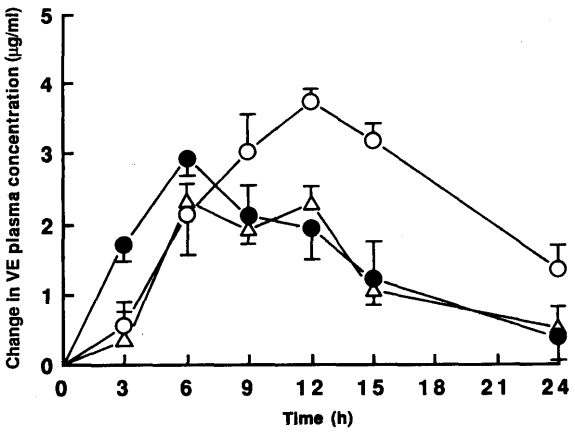

Fig. 2. Changes in Plasma Concentration of *d*-α-Tocopherol (VE) Following Oral Administration of *d*-α-Tocopherol Acetate Preparations in Rats

△, soybean-oil solution; ○, lecithin-dispersed aqueous preparation; ●, polysorbate 80-solubilized aqueous solution. VE plasma concentration just before the administration was subtracted from each determined value. Results are expressed as the mean + or − S.E. of 6 rats.

TABLE II. Bioavailability Parameters for Increased *d*-α-Tocopherol Following Oral Administration of *d*-α-Tocopherol Acetate Preparations in Rats

Dosage form	AUC_{0-24h} (μg·h/ml)	*MRT* (h)	C_{max} (μg/ml)	T_{max} (h)
(1) Soybean-oil solution	29.16 ± 3.57	11.3 ± 0.5	2.66 ± 0.24	8.0 ± 1.0
(2) PS-80-solubilized aqueous solution	35.51 ± 7.49	9.0 ± 1.3	3.05 ± 0.30	7.0 ± 1.0
(3) Lecithin-dispersed aqueous preparation	53.34 ± 4.51[a]	12.9 ± 0.6[b]	3.87 ± 0.24[a]	11.0 ± 1.3[b]

VE plasma concentrations used for the calculation were those shown in Fig. 2. Each value is expressed as the mean ± S.E. of 6 rats. *a*) Significantly different from (1) ($p < 0.01$). *b*) Significantly different from (2) ($p < 0.05$).

body has not been clarified. Based on the assumption that the *MRT* value after i.v. administration of VE is common for the three preparations, the mean absorption time (*MAT*) seems to be longest in the lecithin-dispersed preparation, indicating that VE absorption from the lecithin-dispersed VEA preparation is the slowest of the three. However, since VEA is administered repeatedly in general and VE is generally recognized as a safe compound, the slow absorption and the slight accumulation in the body are not serious problems.

It has been shown that VE is absorbed by a passive diffusion mechanism from mixed micelles consisting of taurocholate, oleic acid and monoolein and that the absorption is predominantly from the middle part of the small intestine.[6] Furthermore, it has been reported that VE is mainly transported *via* the lymphatic route[7] and that the absorption *via* the portal route is only about 8%,[7] though the proportion depends on the dosage forms. Since VE in soybean-oil solution would be absorbed *via* the lymphatic route, the appearance in the plasma is slow. However, the PS-80-solubilized solution lacks components for the formation of the chylomicrons, which are considered to be a carrier for the lymphatic transport of VE. Thus, the early elevation of the plasma concentration observed following administration of the PS-80-solubilized solution might be due to the absorption *via* the portal route. In order to investigate the dosage form dependency of the lymphatic absorption, plasma concentrations of VE following intraduodenal administration of PS-80-solubilized and lecithin-dispersed VEA preparations were examined in thoracic duct fistula rats. As is evident from Fig. 3, the plasma concentration was not elevated by the lecithin-dispersed preparation in the thoracic duct fistula rats, while the PS-80-solubilized preparation could increase the plasma concentration of VE even in the thoracic duct fistula rats. Figure 4 shows the rate of VE transport in the thoracic lymph in the same animals as in Fig. 3. It has been shown that VE is well-absorbed from the MCTG emulsion mainly *via* the lymphatic pathway[2] and that VE absorption from the MCTG emulsion is remarkable in the proximal small intestine,[2] in contrast to the slower formation of mixed micelles and the slower lymphatic transport in the case of the long-chain triglyceride emulsion. Although some lymphatic transport of VE was observed following administration of PS-80-solubilized VEA preparation, the transport was remarkably high in the case of the lecithin-dispersed preparation. MCTG in both preparations might play some role in the lymphatic transport of VE. These results clearly indicate that VE is absorbed from the lecithin-dispersed VEA aqueous preparation *via* the lymphatic route, and agree well with those reported by Gallo-Torres *et al.*[2] The slower and more prolonged absorption of VE from the lecithin-dispersed preparation (Fig. 2) would be due to the lymphatic absorption. It is supposed that the smaller particle size in the lecithin-dispersed preparation in comparison with the oil solution is advantageous for uptake by the intestinal mucosa. Further investigation is necessary to clarify the role of MCTG in the intestinal absorption of the vitamin and its fate thereafter.

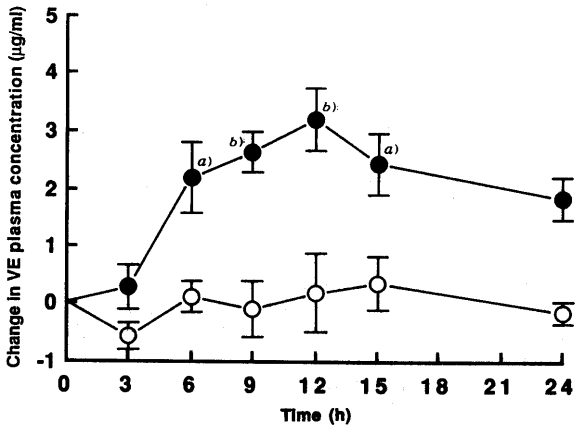

Fig. 3. Changes in Plasma Concentration of *d*-α-Tocopherol (VE) Following Intraduodenal Administration of *d*-α-Tocopherol Acetate Preparations in Thoracic Duct Fistula Rats

○, lecithin-dispersed aqueous preparation (3); ●, polysorbate 80-solubilized aqueous solution (5). VE plasma concentration just before the administration was subtracted from each determined value. Results are expressed as the mean ± S.E. with the number of rats in parentheses. *a*) $p<0.05$. *b*) $p<0.01$.

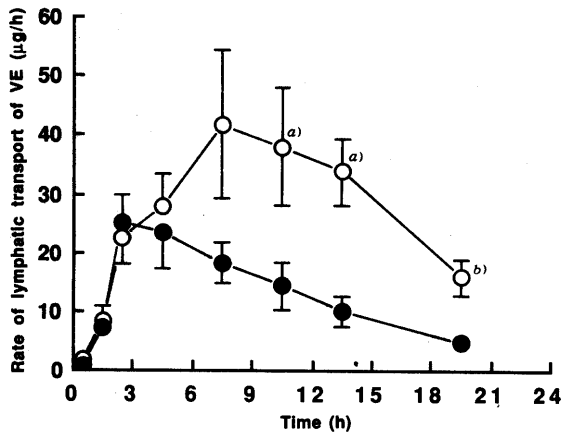

Fig. 4. Rate of *d*-α-Tocopherol (VE) Transport in Thoracic Lymph Following Intraduodenal Administration of *d*-α-Tocopherol Acetate Preparations

Animals and symbols are the same as in Fig. 3. Results are expressed as the mean ± S.E. *a*) $p<0.05$. *b*) $p<0.01$.

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