Intestinal Absorption of Tocopherol in Beagle Dog and Effect of Dosage Form

TADAKAZU TOKUMURA,* a YOSHIHARU MACHIDA,b YUKI TSUSHIMA,c MASANORI KAYANOD and TSUNEJI NAGAI

Tsukuba Research Laboratories, Eisai Co., Ltd., a 1-3 Tokodai 5-chome, Toyosato-machi, Tsukuba-gun, Ibaraki 300-26, Japan, Faculty of Pharmaceutical Sciences, Hoshi University,b Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan, Products Formulation Research Laboratory, Eisai Co., Ltd., c Minami 2-3-14, Honjo-shi, Saitama 367, Japan and Pharmaceutical Research Laboratories, Eisai Co., Ltd., d 1, Takehaya-machi, Kawashima-cho, Hijima-gun, Gifu 483, Japan

(Received February 20, 1987)

Intestinal absorption of d-a-tocopherol from four model preparations was investigated after oral administration to beagle dogs. The bioavailability of d-a-tocopherol from the model preparations was evaluated by determination of the plasma level after oral administration. It was found that the absorption of d-a-tocopherol was not affected by food, and that the difference in dosage forms caused a difference in bioavailability. The tablet showed lower availability than capsules filled with oily solution.

Keywords—d-a-tocopherol; dosage form; oral administration; beagle dog; plasma concentration; bioavailability; HPLC method

A water-insoluble drug is generally considered to be absorbed through the same process as lipids in the gastro-intestinal tract. When a pharmaceutical preparation of a water-insoluble drug is orally administered, most of the drug is absorbed through the following three steps, i.e., dispersion, emulsification by the action of bile, pancreatic juice and peristalsis of the intestinal tract, and formation of mixed micelles. On the other hand, it has been reported that different extents of bioavailability were observed on the oral administration of different preparations of a slightly water-soluble drug. In the case of slightly water-soluble drugs, dissolution from the preparations is considered to be the rate-determining step of absorption. If the micelle formation step in the absorption process of a water-insoluble drug corresponds to the dissolution step of a slightly water-soluble drug, the bioavailability of a water-insoluble drug may depend on the preparation employed. However, few bioavailability studies have been done on water-insoluble drug preparations. One reason for this is considered to be that the effect of physiological factors on the absorption of a water-insoluble drug is larger than that on a water-soluble or slightly soluble drug. This presents a problem in animal experiments to detect differences between preparations of water-insoluble drugs. Further, the rat has been the experimental subject in the majority of absorption studies on water-insoluble drugs, and observations in dogs or humans have been scant. However, a pharmaceutical preparation designed for humans cannot be administered directly to rats. Therefore, we examined the bioavailability of a water-insoluble drug, d-a-tocopherol, by administering several model preparations to beagle dogs, because a pharmaceutical preparation for humans can be administered directly and the dogs have a gallbladder. In addition, the effect of food, which is a
factor affecting the absorption of water-insoluble drugs, was examined. \(d-\alpha\)-Tocopherol is well known to be absorbed through mixed micelle formation. In order to examine the differences of bioavailability four different model preparations, having markedly different properties, of \(d-\alpha\)-tocopherol were prepared and used.

**Experimental**

**Materials** — \(d-\alpha\)-Tocopherol and tocol purified by Eisai Co., Ltd., were used. All other chemicals and solvents used were of analytical reagent grade.

**Absorption Study** — Four male beagle dogs with free access to water were used under fasted and non-fasted conditions. In the former case, the dogs were fasted for 18 h before drug administration and 12 h after drug administration. In the latter, the dogs were fasted from 18 h before drug administration and then given 100 g of Solid Feed DS (Oriental Yeast Co., Ltd., Tokyo, Japan) 1 h before drug administration. After drug administration the dogs were again fasted for 12 h. The concentration of endogenous \(d-\alpha\)-tocopherol in dog plasma was determined under non-fasted conditions. The intervals between administrations were more than one week. The dose of \(d-\alpha\)-tocopherol per dog was 300 mg unless otherwise stated. The following four preparations, A, B, C and D, were administered to the dogs with 30 ml of water.

Preparation A is a capsule containing 300 mg of \(d-\alpha\)-tocopherol dissolved in 600 mg of polyoxyethylene derivative of hydrogenated castor oil (HCO-60) and 600 mg of polyethylene glycol 400 (PEG-400). This preparation forms micelles when added to water, and \(d-\alpha\)-tocopherol is dissolved in water. Preparation B is a capsule containing 300 mg of \(d-\alpha\)-tocopherol dissolved in 150 mg of cottonseed oil and 600 mg of decaglycerin monolaurate. This preparation forms an emulsion when added to water and \(d-\alpha\)-tocopherol is dispersed in the water. Preparation C is a tablet containing 50 mg of \(d-\alpha\)-tocopherol. This tablet was prepared as follows. \(d-\alpha\)-Tocopherol was added to the same weight of silicic acid anhydride. After \(d-\alpha\)-tocopherol was adsorbed, mannitol, polyvinylpyrrolidone K-30 and water were added and the mixture was granulated. The granules were dried for 18 h at 40 °C. Hydroxypropylcellulose and stearic acid were added to the dried granules, and tablets were prepared using a rotary tableting machine (Hata P-13). The weight, hardness and disintegration time in water of these tablets were 254.3 mg, 1.6 kg and 5—6 min, respectively. Preparation D is a capsule containing 300 mg of \(d-\alpha\)-tocopherol dissolved in 390 mg of cottonseed oil.

At given intervals, a 2-ml blood sample was taken from the cephalic vein. The blood sample was centrifuged for 10 min at 3000 rpm. The plasma layer was removed and frozen at —20 °C until analysis. Then 200 µl of plasma, 1 ml of water and 1 ml of ethanol containing 2 µg of tocol as an internal standard were added to a light-resistant glass-stoppered centrifuge tube. The tubes were shaken for 3 min, then 5 ml of hexane was added, and the tubes were shaken for a further 20 min. After centrifugation for 10 min at 3000 rpm, the hexane phase was collected and evaporated to dryness under nitrogen on a water bath. The residue was dissolved in 200 µl of ethanol, and 20 µl of the solution was injected into a Shimadzu LC-5A high performance liquid chromatography (HPLC) instrument. The chromatograph was operated at a flow rate of 1.8 ml/min and the eluate was monitored spectrofluorometrically by using a fluorescence monitor (Shimadzu RF-530). The excitation wavelength and analyzed wavelength were 294 and 340 nm, respectively. A column of Nucleosil C\(_18\) (5 µm in 4.5 mm × 25 cm) and methanol as the mobile phase were used for analysis. A standard curve was prepared by analyzing plasma to which \(d-\alpha\)-tocopherol had been added at various concentrations ranging from 5 to 50 µg/ml.

**Results and Discussion**

**Endogenous \(d-\alpha\)-Tocopherol**

\(d-\alpha\)-Tocopherol, a natural-type tocopherol, exists in blood and plasma of dogs. In order to determine the increase of plasma \(d-\alpha\)-tocopherol concentration after oral administration, it was necessary to subtract endogenous \(d-\alpha\)-tocopherol concentration. Figure 1 shows the time course of plasma concentration of \(d-\alpha\)-tocopherol in non-fasted animals, when \(d-\alpha\)-tocopherol was not administered. The concentrations ranged from 10 to 12 µg/ml. It was found that the \(d-\alpha\)-tocopherol level was approximately constant. From this result, the concentration of endogenous \(d-\alpha\)-tocopherol was considered to be equal to its plasma concentration just before the administration of \(d-\alpha\)-tocopherol. The increase of \(d-\alpha\)-tocopherol after oral administration was calculated by subtracting the concentration of endogenous \(d-\alpha\)-tocopherol from each determined value.

**Dose of \(d-\alpha\)-Tocopherol**

The absorption mechanism of tocopherol from the gastro-intestinal tract is generally
considered to be a simple diffusion. However, there were some reports suggesting the existence of specific proteins participating in the transport and uptake of tocopherol. Further, it was reported that the efficiency of absorption decreased with increase in the dose of tocopherol in humans, and it was also suggested that there might be a saturable process involved in the absorption. Therefore, the effect of dose on the plasma concentration of d-α-tocopherol was examined. Figure 2 shows the plasma concentrations after oral administration of 200 and 300 mg of d-α-tocopherol as preparation A to fasted animals. The AUC_0–24h and C_max of the 200 mg dose were 171.1 ± 39.2 μg·h/ml (mean ± S.E.) and 11.7 ± 2.5 μg/ml, respectively. When these values were compared with the values in the case of the 300 mg dose shown in Table II, AUC_0–24h and C_max were 1/1.4 and 1/1.3, respectively. It was confirmed from this result that the plasma concentration of d-α-tocopherol was proportional to the dose on oral administration. Therefore, the difference of plasma concentrations of d-α-tocopherol at a given dose is considered to be the result of differences between dosage forms or preparations.

**Absorption in the Non-fasted State**

Figure 3 shows the plasma concentration after oral administration of preparations A—D to non-fasted animals. No significant difference was observed among preparations A—D. The bioavailability parameters, AUC_0–24h, C_max, and T_max, as shown in Table I, were also statistically equal among the four preparations. However, the plasma concentration, C_max and AUC_0–24h of preparation C were smaller than those of preparations A, B and D. This tendency might be caused by the difference of dosage forms, i.e., preparation C is a tablet and preparations A, B and D are capsules filled with solution.

**Absorption in the Fasted State**

Figure 4 shows the plasma concentrations of d-α-tocopherol after oral administration of preparations A—D to fasted animals. The plasma level of preparation C is lower than those of preparations A, B and D, and there was a significant difference between preparations B and C at 8 h. Table II shows the bioavailability parameters of preparations A—D in fasted animals. The AUC_0–24h of preparation C was significantly smaller than those of preparations A and B. In the case of C_max, there was a significant difference between preparations B and C. The values of T_max varied among the four preparations, but the reason for this was not clear. This result is similar to that in non-fasted animals. However, the difference among the
preparations was clearer in fasted animals than in non-fasted animals. Therefore, fasted animals should be preferable to detect differences in bioavailabilities among the preparations.

**Effect of Food on Absorption**

Figure 5 compares the bioavailability parameters in non-fasted and fasted animals on the basis of the data in Tables I and II. There is no significant difference between the results in non-fasted and fasted animals for each preparation.

Intestinal absorption of tocopherol, as already described, is considered to be similar to the absorption of lipids. Therefore, it was easy to predict the effect of bile secretion on the absorption of \(d\)-\(\alpha\)-tocopherol. However, no effect of food on the absorption of \(d\)-\(\alpha\)-

---

**Table I. Bioavailability Parameters for Oral Administration of \(d\)-\(\alpha\)-Tocopherol to Non-fasted Dogs**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>(AUC_{0-24\text{h}}) ((\mu)g \cdot h/ml)</th>
<th>(C_{\text{max}}) ((\mu)g/ml)</th>
<th>(T_{\text{max}}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>237.4±21.7</td>
<td>16.2±1.7</td>
<td>6.5±1.0</td>
</tr>
<tr>
<td>B</td>
<td>273.2±72.4</td>
<td>19.0±4.7</td>
<td>8.5±1.5</td>
</tr>
<tr>
<td>C</td>
<td>193.6±54.0</td>
<td>13.0±3.4</td>
<td>9.0±0.6</td>
</tr>
<tr>
<td>D</td>
<td>217.9±39.1</td>
<td>16.4±3.0</td>
<td>8.0±1.4</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. of 4 dogs.

**Table II. Bioavailability Parameters for Oral Administration of \(d\)-\(\alpha\)-Tocopherol to Fasted Dogs**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>(AUC_{0-24\text{h}}) ((\mu)g \cdot h/ml)</th>
<th>(C_{\text{max}}) ((\mu)g/ml)</th>
<th>(T_{\text{max}}) (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>240.4±36.6(^a)</td>
<td>15.1±2.5</td>
<td>10.0±1.2</td>
</tr>
<tr>
<td>B</td>
<td>250.3±10.9(^b)</td>
<td>17.5±0.5(^b)</td>
<td>8.5±1.0</td>
</tr>
<tr>
<td>C</td>
<td>157.7±35.9(^a)</td>
<td>11.4±0.9(^b)</td>
<td>11.5±4.2</td>
</tr>
<tr>
<td>D</td>
<td>217.9±47.4</td>
<td>14.4±3.1</td>
<td>7.3±0.7</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E. of 3—4 dogs. \(^a\) \(p<0.05\). \(^b\) \(p<0.02\).
tocopherol was observed. It is considered from this result that the amount of bile salts in the intestinal tract of fasted animals is enough, since d-α-tocopherol easily forms mixed micelles with bile salts.

Thus, the bioavailability of d-α-tocopherol from the four model preparations was evaluated by the determination of the plasma level of d-α-tocopherol after oral administration. It was found that the absorption of d-α-tocopherol was not affected by food, and that differences of the dosage forms did cause differences of bioavailability. In the case of d-α-tocopherol, a solid dosage form such as preparation C was less effective than solution-type dosage forms such as preparations A, B and D.

**Acknowledgement** The authors are very grateful to Miss Azusa Koseki, Mr. Takeshi Igawa, Mr. Kazuhiro Inouye, Mr. Masafumi Teshigawara and Mr. Yosiyuki Harada for their assistance in the experimental work.

**References and Notes**

1) This work was presented at the 106th Annual Meeting of the Pharmaceutical Society of Japan, Chiba, April 1986.