Evaluation of Bioavailability upon Oral Administration of Phytonadione Preparations in Beagle Dogs

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Bioavailability of phytonadione was investigated after oral administration to beagle dogs. The administrations of phytonadione in a surfactant solution (preparation a) and in an oleic acid solution (preparations b) greatly increased the bioavailability of phytonadione. The AUCs of preparations a and b were about 2.5 times larger than those of commercially available tablets A and C. This result well corresponded to the results of the dissolution test previously reported. The absorption of phytonadione from the gastro-intestinal tract was affected by food and the bioavailability was largely increased under non-fasted conditions. However, a large scatter was observed in the data, and it was found that the evaluation of the bioavailability of phytonadione preparations could not be done in non-fasted animals.

Keywords phytonadione; bioavailability; beagle dog; surfactant solution; oleic acid solution; commercially available tablet

Phytonadione is a water-insoluble and oily drug, and is widely used orally to treat various problems based on deficiency of vitamin K. Many kinds of commercially available preparations were supplied from several pharmaceutical companies. However, the evaluation of bioavailability for those phytonadione pharmaceutical preparations has not been done enough. The absorption process of phytonadione in the gastro-intestinal tract is considered to be the same process as lipids and tocopherol.

In a previous paper,1) we reported that new dissolution test methods, the bile salts method2) and the bead method,3) were applied for the measurement of the dissolution property of phytonadione for 10 preparations (3 different model preparations and 7 different commercially available tablets), and that the difference of dissolution property among those preparations was observed. In that study, a possibility that the two preparations indicating rapid dissolution rate increased the bioavailability of phytonadione was suggested.

It was also reported previously that the bioavailability of d-α-tocopherol could be evaluated by the determination of the plasma level after oral administration to beagle dogs, and that the difference among the preparations was clearer in fasted animals than in non-fasted animals.4) Therefore, we tried to evaluate the bioavailability of phytonadione following oral administration of the two preparations with rapid dissolution rate.

Experimental

Materials Phytonadione (Eisai Co., Ltd.) was used and α-docosanyl-naphthoate was synthesized according to the method reported by Yamano et al.5) All other chemicals and solvents used were of analytical grade.

Phytonadione Preparations Preparation a is a capsule containing 75 mg of phytonadione dissolved in 75 mg of cottonseed oil, 375 mg of decaglycerin monolaurate and 225 mg of Sunsoft No. 818 (Taiyo Kagaku Co., Ltd., Mie, Japan). Preparation b is a capsule containing 75 mg of phytonadione dissolved in 675 mg of oleic acid. These preparations, a and b, were of the same type as preparations C and E in the previous study.5)

Three kinds of commercially available tablets, A, B and C, containing 5 mg of phytonadione were used. Tablet A had the most rapid dissolution rate in the dissolution tests among the three tablets, and the dissolution rate from tablet C was the slowest.1)

Absorption Study Seven male beagle dogs with free access to water were used under fasted or 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 intervals between administrations were more than one week. A dose of phytonadione per dog was 75 mg unless otherwise stated. The drug was administered to dogs with about 30 ml of water. At given intervals, 2 ml blood samples were taken from the forefoot vein. The blood samples were centrifuged for 10 min at 3000 rpm. The plasma layer was removed and frozen at –20 °C until analysis. The determination of plasma phytonadione level was carried out according to the following modified method based on the methods reported by Abe et al.6,7) A 200 μl of plasma, 1 ml of water and 1 ml of ethanol containing 6 μg of α-docosanyl-naphthoate as an internal standard were added to a light-resistant glass-stoppered centrifuge tube. After adding 5 ml of hexane, the tube was shaken for 20 min and centrifuged 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 Hitachi 655 liquid chromatography. The chromatogram was operated at a flow rate of 1.5 ml/min and the eluate was monitored by using a UV monitor (Hitachi 638). The analyzed wavelength was 254 nm. Developsol ODS-3μ (Nomura Science) and methanol were used as a column and mobile phase, respectively. The detection limit of phytonadione in this method was 50 ng/ml.

Results and Discussion

Dose of Phytonadione In order to fix the dose of phytonadione to beagle dogs, a preliminary experiment was performed. This experiment was carried out using one dog at each dose, and tablet B was used because tablet B had the moderate dissolution rate among the commercially available tablets A, B and C. Figure 1 shows the plasma concentrations after oral administration of 30, 60 and 90 mg of phytonadione as tablet B to non-fasted animal. The values of AUC₀–₁₂₇, at 30, 60, and 90 mg were 1443.8, 3797.0 and 15949.7 ng h/ml, respectively. The plasma concentration of phytonadione could be detected within 12 h after drug administration at the doses of 60 and 90 mg, and within 8 h at 30 mg. In the evaluation of phytonadione preparations, the drug is administered orally to fasted animals. In this case, the plasma concentration of phytonadione may decrease largely compared with the result of the preliminary...
study. Therefore, the dose of phytonadione was fixed at 75 mg per animal.

**Effect of Dosage Form** Figure 2 shows the plasma concentration of phytonadione after oral administration of preparations a and b, and two tablets, A and C, to fasted animals. The plasma concentrations of phytonadione of preparation a at 2 and 3 h were significantly higher ($p < 0.01$) than those of tablets A and C, and the plasma concentration of preparation b at 3 h was also significantly higher ($p < 0.01$) than those of tablets A and C. There was no difference of plasma levels between preparations a and b, and between tablets A and C.

Table I shows the bioavailability parameters of preparations a and b, and tablets A and C. The values of $C_{\text{max}}$ of preparations a and b were larger than those of tablets A and C, especially, the $C_{\text{max}}$ of preparation b was significantly larger than those of tablets A and C.

The $T_{\text{max}}$ values of preparations a and b were equal, and the $T_{\text{max}}$ of tablet A was larger than those of preparations a and b, further, the $T_{\text{max}}$ of tablet C was more larger. However, there was no significant difference among these data.

There was no difference in the $AUC_{0-8h}$ values between preparations a and b, and between tablets A and C. The $AUC$s of preparations a and b were about 2.5 times larger than those of tablets A and C. The significant differences between preparation a and tablet A and between preparation a and tablet C were observed. However, there was no significant difference between preparation b and tablets A or C.

The scatter of the data of preparation a was the smallest. For example, the coefficient of variations (CV) of preparation a for $C_{\text{max}}$ and $AUC$ were 28.1% and 26.3%, however, the CVs of preparation b and tablets A and C ranged from 51.8% to 79.6%. This small scatter of preparation a was considered to be caused by the rapid dissolution rate of phytonadione from preparation a. In the comparison of preparations a and b, the mean values of plasma levels and in bioavailability parameters were nearly equal, but the scatter of the data of preparation a was smaller. Therefore, preparation a is better as the dosage form of phytonadione.

These data indicated that the administrations of phytonadione in a surfactant solution (preparation a) and in an oleic acid solution (preparation b) greatly increased the bioavailability of phytonadione. This result corresponds well to the results of the dissolution test previously reported. For example, Fig. 3 shows the relationship between the $AUC$ values and the percent dissolved of phytonadione from each dosage form at 0.5 h in the bead method reported previously. A significant dependence was observed between the $AUC$ values and the data of the

**Table I. Bioavailability Parameters for Oral Administration of Phytonadione to Fasted Dogs**

<table>
<thead>
<tr>
<th>Preparation</th>
<th>$n$</th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>$AUC_{0-8h}$ (ng h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparation a</td>
<td>5</td>
<td>$880.9 \pm 110.8$</td>
<td>$2.40 \pm 0.25$</td>
<td>$3548.2 \pm 416.6$</td>
</tr>
<tr>
<td>Preparation b</td>
<td>5</td>
<td>$1212.3 \pm 431.3$</td>
<td>$2.40 \pm 0.25$</td>
<td>$3396.7 \pm 1138.3$</td>
</tr>
<tr>
<td>Tablet A</td>
<td>7</td>
<td>$373.9 \pm 89.1$</td>
<td>$2.71 \pm 0.36$</td>
<td>$1390.3 \pm 376.3$</td>
</tr>
<tr>
<td>Tablet C</td>
<td>7</td>
<td>$472.6 \pm 92.6$</td>
<td>$3.29 \pm 0.29$</td>
<td>$1396.2 \pm 343.7$</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.  

a) $p < 0.05$, b) $p < 0.01$.  

Fig. 1. Effect of Dose of Phytonadione on Plasma Concentration in Non-fasted Beagle Dogs  
●, 30 mg; ○, 60 mg; ▲, 90 mg.

Fig. 2. Plasma Concentration of Phytonadione Following Oral Administration to Fasted Dogs  
●, preparation a; ○, preparation b; ▲, tablet A; △, tablet C. Each points represents the mean ± S.E. of 5 or 7 dogs.

Fig. 3. Relationship between $AUC$ and the Percent Dissolved of Phytonadione from Each Dosage Form at 0.5 h in Bead Method.  
Each point represents the mean ± S.E. The percent dissolved is the data from the previous paper.  

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dissolution test ($p < 0.05$), which was expressed as a linear relationship with $r = 0.920$. The increase of bioavailability of preparations a and b had been presumed by the dissolution test. This indicates that there is a rate-determination step of absorption of phytanodione in the process of forming micelles by the action of bile salts. Preparations a and b were designed to easily form micelles with bile salts. It seems that this dosage form design causes the increase of bioavailability. From this point of view, the dissolution tests using a bile salt solution, the bile salts method$^2$ and the bead method$^3$ were found to be good methods for the dosage form design of a water-insoluble and/or oily drug.

On the other hand, when the plasma concentrations of phytanodione of tablets A and C were compared with those of the preliminary study, the plasma concentrations were considered to be much lower. From the comparison of experimental conditions, a cause of this phenomenon was estimated to be the effect of food on the absorption. Therefore, the following examination was carried out.

**Effect of Food on Absorption** Figure 4 shows the plasma concentration of phytanodione after oral administration of tablets A, B and C to non-fasted animals. In the comparison of the mean values, the concentration of tablet B at 1 h was lower than those of tablets A and C. However, each value varied widely and the significant difference was not observed among tablets A, B and C. In bioavailability parameters, $C_{\text{max}}$, $T_{\text{max}}$ and $AUC_{0-\text{8hr}}$, as shown in Table II, were also statistically equal among three kinds of tablets because of a large scatter. In addition, the data of tablet C at 75 mg was considered to be reasonable in comparison with the result of the preliminary study.

This result, in which a large scatter was observed in the data, indicates that the influence of food on the absorption of phytanodione is very large, and that the evaluation of the bioavailability of phytanodione preparations must not be done in non-fasted animals. Accordingly, the examination for preparations a and b in non-fasted animals was not tried.

In a comparison of the bioavailability parameters between fasted and non-fasted animals as shown in Tables I and II, the $AUC$ values of tablets A and C were significantly increased under non-fasted conditions ($p < 0.05$). The values of $C_{\text{max}}$ of tablets A and C in the fasted animal were 6.0 and 4.8 times larger than those in the fasted animals. The elongation of $T_{\text{max}}$ was observed in fasted animals compared with non-fasted animals. Especially, a significant difference was observed in the result of tablet A ($p < 0.05$). It was indicated from this result that the absorption of phytanodione from the gastro-intestinal tract was affected by food. This is different from the result of $d$-$\alpha$-tocopherol. In the case of $d$-$\alpha$-tocopherol, the absorption was not affected by food in the same experimental system.$^4$ This difference may be caused by the different interaction between bile and phytanodione or $d$-$\alpha$-tocopherol.

As noted above, the administrations of phytanodione in a surfactant solution (preparation a) and in an oleic acid solution (preparations b) greatly increased the bioavailability of phytanodione. The $AUC$s of preparations a and b were about 2.5 times larger than those of commercially available tablets A and C. This result well corresponded to the results of the dissolution test previously reported.$^1$ The absorption of phytanodione from the gastro-intestinal tract was affected by food and the bioavailability was largely increased under non-fasted conditions. However, the large scatter was observed in the data, and it was found that the evaluation of the bioavailability of phytanodione preparations could not be done in non-fasted animals.

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**References**