J. Pharm. Pharmacol. 1999, 51: 1375–1380
Received March 26, 1999
Accepted July 13, 1999

Absorption of Vitamin K_2 by Dogs after Oral Administration of a Soft Gelatin Capsule Formulation Containing a New Emulsion-type Vehicle

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Abstract

This study has evaluated the performance of a newly developed vehicle for administration of a drug in a soft gelatin capsule. The absorption of vitamin K_2 in dogs after oral administration of the vitamin in a soft gelatin capsule containing the newly developed vehicle was compared with absorption after administration of a control formulation prepared by encapsulating the contents of a commercially available vitamin K_2 capsule (Glakay capsules 15 mg) in the same type of soft gelatin.

Under non-fasted conditions the profile of the plasma concentration of vitamin K_2 against time for the test formulation was comparable with that for the control formulation in non-fasted dogs. Under fasted conditions, however, both the maximum concentration (C_{max}) and the area under the plot of concentration against time (AUC) were significantly smaller for the test formulation than for the control formulation. The C_{max} and AUC for the test formulation were about 10 times larger for non-fasted dogs than for fasted dogs whereas values for the control formulation were about twice as large. These results suggest that both formulations might require the presence of food or digestive fluid components, or both, for better absorption of vitamin K_2. It seems that although the performances of the test and control formulations were comparable in the presence of these components, the control formulation works better in their absence. It should be also noted that, in contrast with the results from the absorption tests, the dispersibility of the test vehicle in water was much better than that of the control vehicle. This suggests that dispersibility does not significantly affect vitamin K_2 absorption.

In conclusion, although the new vehicle did not perform better than the control vehicle in terms of vitamin K_2 absorption, the performance of the control formulation was comparable for non-fasted dogs. Because the new vehicle contains considerably less surfactant than the vehicles currently used in soft gelatin capsules, it could be a safer alternative for use under non-fasted conditions.

The method of drug dosage used in preclinical studies has recently become an important toxicokinetic issue, because it often influences drug absorption. The soft gelatin capsule has an advantage over other dosage forms, such as granule, tablet and hard gelatin capsule, because the encapsulated solution can be used in both preclinical and clinical studies, thereby minimizing potential dosage form-related discrepancies between these.

A soft gelatin capsule encapsulates a drug in a water-soluble non-aqueous solution, oil solution, or suspension. It is well known that soft gelatin capsules can improve the bioavailability of a drug (Ghirardi et al 1977; Lesko et al 1979; Yamashita et al 1979; Bateman & Uccellini 1984) by accel-

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erating disintegration, dispersion and dissolution in the gastrointestinal tract, and can also improve drug stability because of poor permeability to oxygen (Hom et al 1975).

However, the absorption of oil-dissolved and hydrophobic drugs is known to be poor or erratic, or both, because of poor miscibility or dissolution in the aqueous environment of the gastrointestinal tract. To overcome these problems, self-emulsifying drug delivery systems (Pouton 1985; Craig et al 1993; Shah et al 1994) and microemulsions (Ritschel et al 1990; Ritschel 1991) have recently been developed. However, these newly developed formulations require a high surfactant content (approximately 30–60% of the formulation), which can damage Kupfer cells (Nadai et al 1972, 1975) and unexpectedly enhance the absorption of a simultaneously administered drug.

We have therefore developed a new oil-in-water emulsion-type vehicle for use in soft gelatin capsules (Amemiya et al 1998a, b, 1999) with a low content (3·0%) of the surfactant poly(oxyethylene(20)cetyl ether) (BC-20TX). We have previously found (unpublished work) that the new vehicle, containing vitamin K_2 as a model hydrophobic drug, is sufficiently stable for preclinical and clinical studies, in terms of the physicochemical stability of the vehicle and chemical stability of the vitamin K_2.

To examine further the performance of the new vehicle, a soft gelatin capsule containing the new vehicle and vitamin K_2 was administered orally to dogs. The absorption of vitamin K_2 was evaluated and compared with absorption after oral administration of the same type of capsule containing the contents of a commercially available vitamin K_2 capsule (Glakay capsules, 15 mg), which is formulated with a higher surfactant content (47%).

Materials and Methods

Chemicals and reagents

Menatetrenone (vitamin K_2) was purchased from Nisshin Flour Milling (Japan) and phytonadione (Vitamin K_1) from Wako Pure Chemical Industries (Japan). Poly(ethylene glycol) 400 (JP; PEG 400) and poly(ethylene glycol) 6000 (JP; PEG 6000) were purchased from NOF (Japan), medium-chain triglyceride (JPE; Miglyol 810) from Hüls (Germany) and poly(oxyethylene(20)cetyl ether) (BC-20TX) from Nikko Chemicals (Japan). Reagents (analytical grade) for buffer solutions, and other solvents (HPLC-grade), were purchased from Nacalai Tesque (Japan). Glakay capsules, 15 mg, were purchased from Eisai (Japan).

Animals

Four beagles, male, 12–14 kg, were obtained from Fuji Biomedix (Japan) and Toyo Beagle Oriental Maruichi Kyodo (Japan). They were separately housed in stainless steel cages, fed once daily in the morning, and had free access to water. Dogs required in the fasted condition were not fed on the days of experiments (period of fasting > 15 h); dogs required in the non-fasted condition were fed 1 h before dosing.

Formulation

The internal solution of the test formulation was prepared from PEG 400 (74·69%), PEG 6000 (2·31%), Miglyol 810 (5·0%), vitamin K_2 (5·0%), BC-20TX (3·0%) and purified water (10·0%). Materials were weighed in a stainless steel beaker, heated to 60°C (approx.) in a water bath, and then mixed (15 000 rev min^{-1} for 10 min) by use of a high-speed mixer (Cell-master CM-100; SMT, Japan) followed by homogenization (10 000 psig; five treatments) with a high-pressure homogenizer (Mini-Lab 8.30H; Rannie, Denmark), as described in our previous reports (Amemiya et al 1998a, 1999). The test formulation was prepared by encapsulating 900 mg (approx.) of the solution in a soft gelatin capsule prepared in our laboratory which was then sealed with gelatin solution. The control formulation was prepared by encapsulating vitamin K_2 solution (15%; 300 mg approx.) obtained from Glakay capsules (15 mg).

Evaluation of dispersibility

The solution of vitamin K_2 from each formulation was put on an aluminium foil plate (5 mm diam., approx.) in water (900 mL; $37 \pm 0{\cdot}5$°C) in an apparatus for the paddle dissolution test (JP13), and dispersed in water by rotation of the paddle at 100 rev min^{-1}. Dispersibility was evaluated by measuring the particle size distribution of the dispersed sample 5 and 30 min after initiating dispersion, by means of a laser diffraction particle-size analyser (SALD-2000A; Shimadzu, Japan).

Evaluation of vitamin K_2 absorption

The study was performed by means of a cross-over design; all the dogs were used in both the fasted and non-fasted condition. The interval between doses was 4 weeks and no carry-over effect was observed. Blood samples (2 mL) were obtained from the cephalic vein 0·5, 1, 2, 3, 4, 6, 8 and 24 h

after oral administration of the test or control formulation (vitamin K_2 dose 4 mg kg^{-1}) with water (20 mL, approx.). The blood was immediately centrifuged to obtain plasma, which was stored in amber glass tubes at −40°C until analysis. To prepare calibration curves for determination of vitamin K_2 concentrations, blood (20 mL) was obtained from each dog a week before each experiment. All plasma samples obtained from the blood samples were pooled before preparing calibration curves.

Analysis of Vitamin K_2 in dog plasma

Vitamin K_2 was quantified by high-performance liquid chromatography (HPLC) by means of a Shimadzu (Japan) LC-6A autosampling and solvent-delivery system equipped with a Shimadzu RF-535 fluorescence spectrophotometer (λ_{ex} 254 nm, λ_{em} 430 nm) and a Shimadzu LC10 data analysis system. Compounds were separated on a 250 mm × 4·6 mm i.d., 5-μm particle, Wakosil-II 5C18-HG ODS column (Wako, Japan), maintained at 40°C, followed by conversion of vitamin K_2 to a hydroquinone derivative (for detection of fluorescence) on a reducing column (RC-10-3, 30 mm × 4 mm i.d.; Irika, Japan), according to the method reported by Shino (1988). The mobile phase was ethanol–methanol–water, 50 : 47 : 3. This method of assay of vitamin K_2 had excellent resolution, with no interfering peaks.

Phytonadione (vitamin K_1; 100 ng) was added as internal standard to an amber glass tube (1·6 mL) containing plasma (200 μL). After addition of phosphate buffer (pH 8·0, 0·2 M; 1 mL), each sample was extracted with isopropyl alcohol–*n*-hexane (8 : 92; 2 × 4 mL) and the organic extracts were transferred to another amber glass tube. Solvent was evaporated under nitrogen at 40°C and the residue was dissolved in the mobile phase. The volume of mobile phase used was 0·2 mL if the plasma vitamin K_2 content was 0–0·5 μg mL^{-1} or 2·0 mL if the plasma vitamin K_2 content was 0·5–10 μg mL^{-1}. Samples (10 μL) were then analysed by HPLC.

Separate calibration curves were prepared for the concentration ranges 0–0·5 μg mL^{-1} and 0·5–10 μg mL^{-1} of vitamin K_2 in plasma. The linearity of the calibration curves was excellent in each assay ($r > 0·999$). No food-derived vitamin K_2 was detected in plasma.

Estimation of pharmacokinetic parameters

The maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined directly from the measured concentrations. The area under the plot of concentration against time (AUC) was calculated by the trapezoidal method, using plasma concentrations up to 24 h after dosing.

Results

Plasma concentrations of vitamin K_2

After oral administration of the test formulation to non-fasted dogs the plasma concentration of vitamin K_2 rose rapidly and reached a C_{max} of 2·91 μg mL^{-1} after 1 h only (Figure 1, Table 1), suggesting rapid absorption of vitamin K_2. The profile of plasma concentration against time for the control formulation was comparable with that for the test formulation, indicating similar rates and extents of vitamin K_2 absorption for these two formulations. Although the values of C_{max} and AUC were 60–80% (approx.) larger for the control formulation than for the test formulation, the differences did not reach statistical significance.

Under the fasted condition, however, plasma concentrations were lower for the test formulation than for the control formulation throughout the experimental period (Figure 2), resulting in significantly smaller C_{max} and AUC values for the test formulation (Table 1). However, T_{max} values were

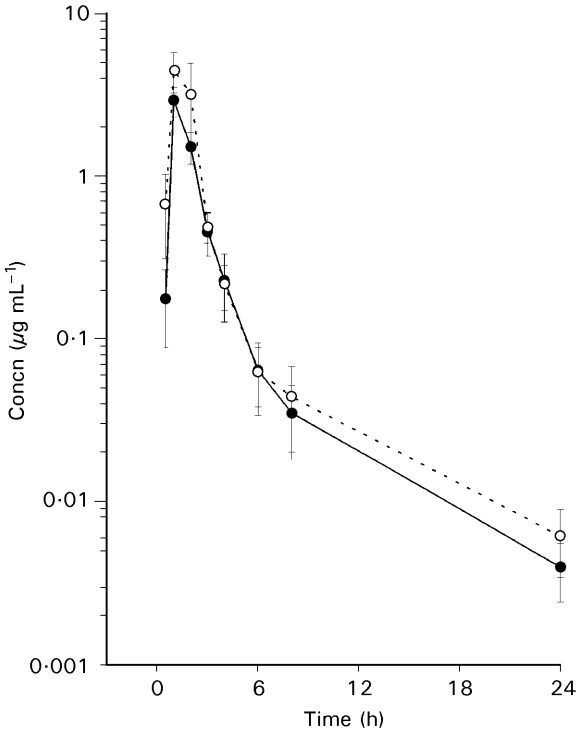

Figure 1. Plasma concentrations of vitamin K_2 in non-fasted dogs. Test (●) and control (○) formulations were administered orally; the dose of vitamin K_2 was 4 mg kg^{-1}.

Table 1. Pharmacokinetic parameters of vitamin K_2 in dogs.

		Time to maximum concn (h)	Maximum concn ($\mu g\,mL^{-1}$)	$AUC_{0-24\,h}$ ($\mu g\,h\,mL^{-1}$)
Test formulation	Non-fasted	1·00 ± 0·00	2·91 ± 0·59*	5·07 ± 0·73** (29·7%)
	Fasted	1·50 ± 0·29	0·31 ± 0·04†	0·60 ± 0·10†† (36·4%)
Control formulation	Non-fasted	1·25 ± 0·25	5·31 ± 1·58	8·26 ± 2·11 (48·9%)
	Fasted	1·50 ± 0·29	2·31 ± 0·42	3·53 ± 0·36 (19·9%)
Glakay capsules[a]	Non-fasted (n = 9)	4·90 ± 1·30	2·05 ± 0·91	2·90 ± 0·80 (82·8%)
	Fasted (n = 3)	2·00 ± 0·00	0·39 ± 0·10	0·75 ± 0·20 (46·7%)

AUC is the area under the plasma concentration–time curve. Test and control formulations were administered orally; the dose of vitamin K_2 was 4 mg kg^{-1}. Data are means ± s.e.m. (n = 4); values in parentheses are coefficients of variation (CV%). *$P < 0.01$, **$P < 0.001$ compared with the test formulation under fasted condition. †$P < 0.01$, ††$P < 0.001$ compared with the control formulation under fasted condition.[a]From Sano et al (1993); these AUC values are for 0 to 10 h after administration.

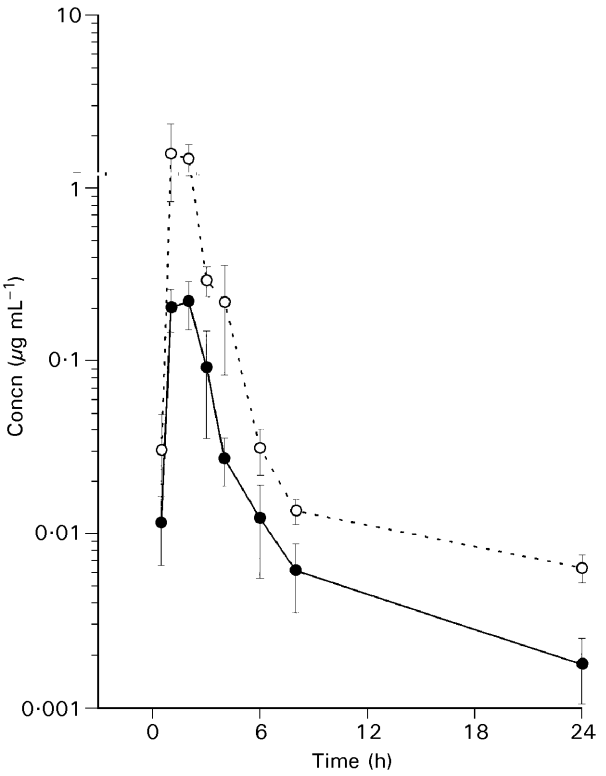

Figure 2. Plasma concentrations of vitamin K_2 in fasted dogs. Test (●) and control (○) formulations were administered orally; the dose of vitamin K_2 was 4 mg kg^{-1}.

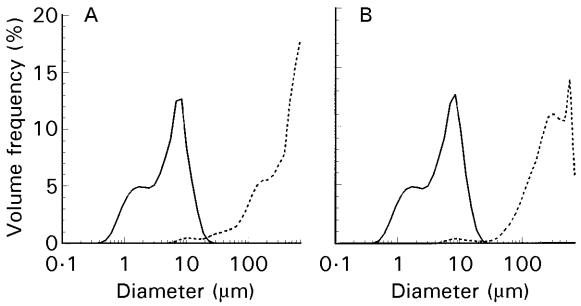

Figure 3. Dispersibility of capsule contents in water. Particle-size distribution was measured A. 5 min and B. 30 min after initiating dispersion of the inner contents of test (—) and control (·····) formulations.

comparable for the two formulations suggesting that absorption was better for the test formulation of vitamin K_2.

Both C_{max} and AUC for the test formulation were 10 times (approx.) larger under the non-fasted condition than under the fasted condition (Table 1). C_{max} and AUC for the control formulation were twice as large (approx.) under the non-fasted condition than under the fasted condition although the difference was not statistically significant. T_{max} for both formulations was not affected by the feeding condition.

These results suggest that both formulations might require food or digestive fluid components, or both, which are more abundant in the non-fasted condition, for better absorption of vitamin K_2. It seems that although the performances of both formations are comparable in the presence of food or digestive fluid, the control formulation works better in their absence.

Dispersibility

In an effort to elucidate the mechanism leading to the difference between the performances of the test and control formulations, the dispersibility of test and control solutions was evaluated by measuring the particle-size distribution by means of a dissolution testing assembly. The test solution was finely dispersed shortly (5 min) after initiating dis-

persion (Figure 3A) and the particle-size distribution profile was unchanged after 30 min (Figure 3B). The control solution was less finely dispersed than the test solution after both 5 and 30 min (Figure 3). Thus, the dispersibility of the test solution was found to be better than that of the control solution, in contrast with results from the vitamin K_2 absorption test.

Discussion

Gastrointestinal absorption of vitamin K_2 can be greatly improved by bile salts, endogenous surfactants contained in the digestive fluid, presumably because of enhanced dissolution of this highly hydrophobic drug as a result of the formation of micelles (Ishii et al 1995; Uematsu et al 1996). Therefore, it is likely that bile salts are the major components of food or digestive fluid that improved the absorption of vitamin K_2 in this study.

Under the fasted condition, when food and digestive-fluid components were inadequate, vitamin K_2 absorption was much greater for the control formulation than for the test formulation (Figure 2, Table 1), presumably because the control formulation contained more surfactant (47%) than the test formulation (3%) and resulted in more efficient dissolution of vitamin K_2. Under the non-fasted condition, rich in food and digestive fluid components, however, vitamin K_2 absorption for both formulations was comparable (Figure 1, Table 1). Thus, the test formulation (or the new vehicle) was found to work as effectively as the control formulation under the non-fasted condition. The new vehicle was formulated with much less surfactant than usual, because surfactants can adversely affect the body. The new vehicle might be a safer alternative for use under the non-fasted condition.

In a study by Sano et al (1993), absorption of vitamin K_2 after oral administration of Glakay capsules to dogs was comparable with the current results for the test formulation rather than the control formulation under both non-fasted and fasted conditions (Table 1). Because the same internal solution was used for the Glakay capsules and the control formulation, the different absorption of vitamin K_2 might be because of a difference between the shell formulae of the soft gelatin capsules. The CV value was smaller for the control formulation than for Glakay capsules under the non-fasted and fasted conditions, indicating that the soft gelatin capsule used in the current study might be better than the Glakay capsules in achieving not only larger but also less erratic absorption of vitamin K_2. The CV value for the test formulation was even smaller than that for the control formulation under the non-fasted condition suggesting that the combination of the new vehicle and our soft gelatin capsule might be more effective in achieving less erratic absorption, assuming that the new vehicle is a safer alternative.

It is well known that the absorption of oil-dissolved or hydrophobic drugs is influenced by the combination and component ratio of surfactants and oils in a vehicle (Ritschel et al 1990; Ritschel 1991; Shah et al 1994). In a previous study we found that a combination of surfactants (poly(ethylene glycol) (40 ethylene oxide) monostearate or poly(ethylene glycol) (60) sorbit tetraoleate) and oils (propylene glycol dicaprylate or soybean oil) can give homogeneous and viscous white gels similar to the vehicle in the current study (Amemiya et al 1998a). A detailed investigation of the effects of the combination and the component ratio of these surfactants and oils on the absorption of vitamin K_2 will be a subject of future investigation. Testing of drugs other than vitamin K_2 in conjunction with the new vehicle, is also an issue to be considered in the future.

In conclusion, although the test formulation using the new vehicle could not outperform the control formulation in terms of vitamin K_2 absorption, its performance was comparable with the control formulation in non-fasted dogs. The new vehicle is prepared with very little surfactant in comparison with currently available vehicles for soft gelatin capsules, because surfactant is believed to cause adverse effects. Therefore, it might be a safer alternative for use under non-fasted conditions.

Acknowledgements

The authors thank R. P. Scherer K. K. and Mr S. Kato (president of R. P. Scherer K. K.) for financial assistance throughout this investigation.

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