Pharmacokinetic Profiles of Coenzyme Q_{10}: Absorption of Three Different Oral Formulations in Rats

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Pharmacokinetics and absorption profiles of coenzymeQ_{10} (CoQ_{10}) from three different oral formulations were evaluated in rats. For the intravenous concentration-time data, a two-compartment open model fitted well. There were no significant changes in the values of the elimination rate constant at the terminal phase, and the half-life of CoQ_{10} was estimated to be 7 to 8 hr. The values of intravenous area under the plasma concentration-time curve up to infinity (AUC_∞) increased with a rise in CoQ_{10} dose (0.025 to 2.5 mg/kg); however, the AUC_∞ showed a nonlinear relationship with the administered dose. The total body clearance (CL_{tot}) increased with a rise in the intravenous dose of CoQ_{10}. The value of CL_{tot} increased in proportion to the intravenous dose. Three different formulations of CoQ_{10} [olive oil solution (control), sub-nanosize particles and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS)-emulsion] were tested in rats. An appropriate compartment model wasn't adapted to the concentration-time data from orally administered CoQ_{10} formulations because plasma concentrations of CoQ_{10} from 10 to 24 hr after administration were markedly increased for all formulations tested. The TPGS-emulsion showed a significantly higher AUC_{0-24} value and absorption rate (Fa) than the other formulations (AUC_{0-24}, 18876 ± 6225 ng · h/ml; Fa, 0.15%). There was no difference in the values of AUC_{0-24} and Fa between the control and subnano-particle formulations. After intraloop administration of CoQ_{10} in the olive oil formulation, there were no significant differences in the plasma concentration of CoQ_{10}, and the residual amounts of CoQ_{10} in the different parts of the intestinal loop (upper jejunum, lower jejunum, ileum) at the end of experiment were almost the same. These observations indicate that the pharmacokinetics of CoQ_{10} are nonlinear, and suggest the existence of a deep compartment for CoQ_{10} accumulation in the intestine. Absorption of CoQ_{10} from the intestine was very poor; however, a higher plasma concentration of CoQ_{10} was achieved by an emulsion formulation using TPGS.

Key words —— coenzymeQ_{10}, oral absorption, bioavailability, pharmaceutics, pharmacokinetics

INTRODUCTION

There is growing interest in the use of coenzymeQ_{10} (CoQ_{10}) as a nutritional supplement. CoQ_{10} is a fat-soluble, vitamin-like benzoquinone compound that functions primarily as an antioxidant, a membrane stabilizer, and a cofactor in the oxidative phosphorylation process that leads to the production of adenosine triphosphate (ATP) in its reduced form.[1–3] CoQ_{10} is widely consumed as a food supplement because of its status as an important nutrient for maintaining human health. The rationale for the use of CoQ_{10} as a medical agent for treating cardiovascular diseases is based on its fundamental role in mitochondrial function and cellular bioenergetics.[4,5] With increasing age, the level of CoQ_{10} synthesis is reduced, resulting in lower plasma levels of CoQ_{10} in elderly people.[6]

The absorption of compounds from the gastrointestinal tract is one of the most important determinants of oral bioavailability. Essentially, the oral absorption of highly water-insoluble drugs is frequently limited by poor intestinal-wall permeability. Supplementary CoQ_{10} is commonly provided as an oily formulation for oral use; however, the intestinal absorption of supplementary CoQ_{10} is slow and limited owing to its hydrophobicity and large molecular weight.[7] Moreover, the absorption of orally administered supplementary CoQ_{10} can be

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enhanced by interactions with food or food components.[4] There are many reports on investigations of the pharmacokinetic profile of CoQ_{10} after oral administration.[8–12] However, the pharmacokinetic results in these reports are inconclusive, and are not accompanied by pharmacokinetic data for intravenous administration of CoQ_{10}.

The objectives of this study were firstly to elucidate the pharmacokinetic properties of CoQ_{10} intravenously administered at various doses, and secondly to seek formulations that optimize the intestinal absorption of CoQ_{10} after oral administration.

MATERIALS AND METHODS

Materials —— CoQ_{10} powder was kindly supplied by Morishita-Jintan Co., Ltd. (Osaka, Japan). D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was obtained from the Peboc Division of Eastman Chemical Ltd. (Llangefni, U.K.). All other chemicals were of reagent grade, and were used without further purification.

Animals —— Male Wistar rats (300–350 g) were procured from Nippon SLC Co., Ltd. (Hamamatsu, Japan). All animal experiments were performed in accordance with the guidelines for animal experimentation of Doshisha Women's College of Liberal Arts, Pharmaceutical Division and the Federal Requirements for Animal Studies. The rats had free access to food and water, and were housed in a temperature-controlled facility (22 ± 2°C) with a 12 hr light/dark cycle for at least one week prior to the experiment.

Preparation of Standard and Test Solutions —— The standard stock solutions of CoQ_{10} were prepared by dissolving in *n*-hexane at a final concentration of 500 μg/ml, and were then stored at −20°C in the dark. Working standards for a calibration curve were prepared by diluting the standard stock solution with methanol at various concentrations. The calibration curve samples were prepared by adding known amounts of the working standards to plasma or dialysate at a volume ratio of 5 : 50. The test solutions of CoQ_{10} for intravenous administration were prepared by dissolving CoQ_{10} in a vehicle composed of 5% ethanol, 5% Cremophor® EL, and 5% dimethyl sulfoxide in deionized water at a final concentration of 0.025–2.5 mg/kg. The formulations for oral administration were provided by three different types of formula as shown in Table 1. Formulation A is a control prepared by dissolving CoQ_{10} in olive oil. Formulation B is a water suspension of sub-nano particles (0.4–8.4 μm), which was prepared by a Nanomizer TL-1500 (Tokai Co. Ltd., Tokyo, Japan). Formulation C is an oil-in-water (O/W)-type emulsion using TPGS as an active surfactant, which was prepared by an ultrasonic method. All formulations included 30 mg of CoQ_{10} per ml.

Intravenous Administration Study —— Rats were fasted for 16–18 hr prior to the experiment, although water was provided ad libitum. Then, the rats were anesthetized by intraperitoneal administration of urethane (1.0 g/kg), and placed in a supine position on a surgical table under an incandescent lamp to maintain body temperature at 37°C. Various test solutions of CoQ_{10} (0.025–2.5 mg/kg) were administered intravenously to the left jugular vein. Blood samples of 0.12 ml were collected from the right jugular vein at 10, 20, 30 min, 1, 2, 3, 4, and 6 hr after CoQ_{10} administration. The blank blood samples were taken 5 min prior to the administration of the test solutions. The blood

Table 1. Formulation of the Test Preparations Containing CoQ_{10} Used in This Study

Formulation	Characteristics	Preparation
A: Control	CoQ_{10} is dissolved in olive oil	CoQ_{10} (150 mg) is dissolved in 5 ml of olive oil that is prewarmed at 37°C
B: Sub-nanosize particle suspension	CoQ_{10} is crushed to sub-nanosize particles by Nanomizer Mark-II	CoQ_{10} (1.5 g) is suspended in water including 0.1% Tween 80, and is crushed using the collision power of Nanomizer Mark-II
C: O/W-type emulsion	A vitamin E derivative, TPGS is used as an emulsifier	To TPGS (1.04 g) dissolved in 8 ml of water, CoQ_{10} (300 mg), and 2 ml of olive oil are added, and then the mixture is homogenized

Final concentration of CoQ_{10} in each formulation was set at 30 mg/ml.

samples were collected in heparinized tubes, and plasma was then obtained from whole blood by centrifugation at 12000 × *g* for 15 min at 4°C, and stored at −80°C until analysis.

Oral Administration Study —— Rats were fasted for 16–18 hr prior to the experiment, although water was provided ad libitum. Then, the rats were anesthetized by intraperitoneal administration of urethane (1.0 g/kg), and placed in a supine position on a surgical table under an incandescent lamp to maintain body temperature at 37°C. Rats received CoQ_{10} formulations orally at doses of 75 mg/kg through a stainless-steel needle. Blood samples of 0.12 ml were collected from the right jugular vein at 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hr after CoQ_{10} administration. The blank blood samples were taken 5 min prior to the oral administration of the test formulations. The blood samples were collected in heparinized tubes, and plasma was then obtained from whole blood by centrifugation at 12000 × *g* for 15 min at 4°C and stored at −80°C until analysis.

***In Situ* Intraloop Administration Method** —— Rats were fasted for 16–18 hr prior to the experiment, although water was provided ad libitum. Then, the rats were anesthetized by intraperitoneal administration of urethane (1.0 g/kg), and placed in a supine position on a surgical table under an incandescent lamp to maintain body temperature at 37°C. A midline longitudinal abdominal incision was made, and an inlet or outlet silicon tube (4 mm *id.*) was placed at the upper jejunum, the lower jejunum, and the ileum to make a 15-cm loop. Then, the loop was flushed 3 times with prewarmed (37°C) phosphate buffered saline containing 25 mM glucose (pH 7.4). The test solution of CoQ_{10} (30 mg/ml in olive oil) was administered to the intestinal loop at a final dose of 75 mg/kg. Blood samples of 0.12 ml were collected from the left jugular vein at 1, 2, 3, 4, 5, and 6 hr after CoQ_{10} administration. At the end of the experiment, the contents of the intestinal loop were immediately removed, and the amount of CoQ_{10} remaining in the loop was measured. Plasma was then separated 12000 × *g*, 15 min, 4°C) and stored at −80°C until analysis.

Drug Assay —— CoQ_{10} in plasma or the intestine was extracted by placing 50 μl of the samples into 1.5 ml polyoxyethylene centrifuge tubes, adding 5 μl of cyclosporine methanol solution (10 μg/ml) as an internal standard, and mixing vigorously for 30 sec. Then, 100 μl of 2% (w/v) $ZnSO_4$ in 50% (v/v) 1-propanol solution was added to precipitate proteins. The mixture was mixed for 10 min, then 0.5 ml of *n*-hexane was added to extract CoQ_{10}. The mixture was again mixed for 10 min, and then centrifuged at 12000 × *g* for 3 min. The supernatant was decanted into a glass test tube, and then evaporated until dry in an evaporator for 30 min at 45°C. The residue was reconstituted in 100 μl of methanol-hexane mixture at a ratio of 95 : 5, and then 20 μl was injected into a liquid chromatography–tandem mass spectrometer (LC-MS-MS) system. The LC-MS-MS analysis was carried out using a high-performance liquid chromatography (HPLC) system consisting of an LC20AD quaternary pump (Shimadzu, Kyoto, Japan) equipped with a vacuum degasser and a SIL 20 A auto sampler with a 100 μl loop (Shimadzu) interfaced with a triple-quadrupole tandem mass spectrometer (Applied Biosystems/MDS Sciex, Burlington, Canada). CoQ_{10} and cyclosporine were separated on a Cosmosil sorb 5 μm column (2.0 mm in diameter × 50 mm, 5C18-AR-II). The mobile phase, which consists of 100% methanol containing 5 mM ammonium formate acid, was degassed before use. The sample was delivered with a flow rate of 0.4 ml/min at a column temperature of 40°C, with each analysis lasting 8.0 min. The mass spectrometer was operated in the turbo ion spray mode with positive-ion detection. The flow rate of nebulizer gas, curtain gas and collision gas were set at 8, 10, and 6 l/min, respectively, and the ion spray voltage and temperature were set at 5500 V and 400°C, respectively. The declustering potential, the focusing potential, the entrance potential, the collision energy, and the collision cell exit potential were set at 86, 200, 10, 27, and 34 V, respectively. Multiple-reaction monitoring analysis was performed with the transition m/z 880.7 for CoQ_{10} and m/z 1219.9 for cyclosporine. All raw data were processed with Analyst Software, version 1.4.1. Taking the peak area ratio of CoQ_{10} against the internal standard, the calibration curves of CoQ_{10} were made in plasma or intestinal contents without CoQ_{10}. The retention times for CoQ_{10} and the internal standard were 4.98 and 0.49 min, respectively, and all separation was completed within 8.0 min. The calibration curves of CoQ_{10} were linear and passed through the origin with correlation coefficients of 0.99 or above. The limit of detection for CoQ_{10} was 0.005 μg/ml.

Pharmacokinetic Analysis —— For intravenous concentration-time data, a two-compartment open model was applied, that is $C_p = A \cdot e^{-\alpha \cdot t} + B \cdot e^{-\beta \cdot t}$, where A, B, α, and β represent hybrid model parameters, and the concentration versus time data of

CoQ_{10} for each rat was fitted to this model using a nonlinear least squares program MULTI, to estimate the value for A, B, α, and β.[13] Then, the rate constants between central and peripheral compartments (k_{12}, k_{21} and k_{10}) were calculated. The area under the plasma concentration-time curve up to the final time (t) (AUC_{0-t}) was calculated using a linear trapezoidal rule. The elimination rate constant (λ_z) was estimated analyzing the terminal linear segment of the log serum concentration-time data, followed by extrapolation to infinity ($AUC_{0-\infty}$) by adding the value of C_{plast}/λ_z to AUC_{0-t}, where C_{plast} is the final measurable plasma concentration. The elimination half-life ($T_{1/2, \lambda_z}$) was calculated from dividing ln2 by λ_z. The volume of distribution at the central compartment (V_1) was calculated as follows: $V_1 = D/(A+B)$, where D represents intravenous dose. The total body clearance (CL_{tot}) was calculated by $k_{10} \cdot V_1$, and the volume of distribution at a steady state (V_{dss}) was calculated by $V_1 \cdot (1+k_{12}/k_{21})$. The fraction of drug absorbed *in vivo* (Fa) was determined by the Loo-Riegelman method[14] using free deconvolution software (DECONV.xls, D3 Institute, Tokyo, Japan) by calculating on integrating-weight function based on an input one (two-compartment open model for i.v.) and oral data, where the values of A, B, α, and β were utilized.

Statistical Analysis —— Statistical analysis was performed by using the software STATCEL for Windows (OMS Co., Ltd., Tokyo, Japan). All values are expressed as the mean ± standard error of the mean (SEM). Statistical differences of the means were considered significant when $p < 0.05$ by one-way analysis of variance (ANOVA) followed by Turkey's multiple range test.

RESULTS

In the intravenous administration study, four different intravenous doses of CoQ_{10} were tested on rats. Figure 1 shows the plasma concentration-time curves of CoQ_{10} after intravenous administration. The corresponding pharmacokinetic parameters are listed in Table 2. A two-compartment open model fitted well with the intravenous administration data. There were no significant changes in the values of the elimination rate constant at terminal phase, λ_z. The half-life, $T_{1/2, \lambda_z}$, was estimated to be 7–8 hr.

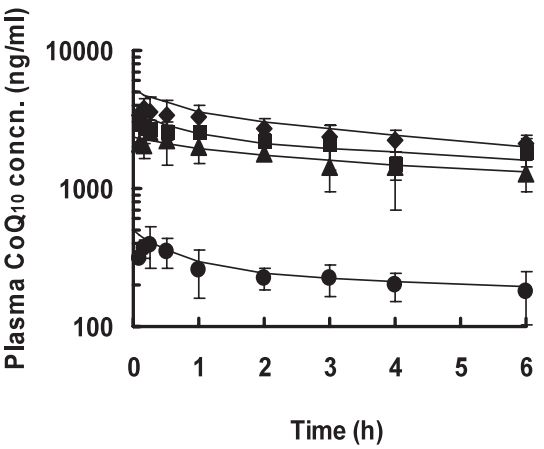

Fig. 1. Plasma CoQ_{10} Concentration Versus Time Curves after Intravenous Administration

The test solutions for intravenous administration include 5% ethanol, 5% Cremophr® EL, and 5% dimethyl sulfoxide in 0.9% saline at a final CoQ_{10} concentration of 0.025–2.5 mg/kg. Key: ●, 0.025 mg/kg; ▲, 0.25 mg/kg; ■, 1.25 mg/kg; ◆, 2.5 mg/kg. Each symbol with bars represents the mean ± S.E. of 4 to 6 rats.

Table 2. Pharmacokinetic Parameters of CoQ_{10} after Intravenous Administration at Various Doses in Rats

IV dose	mg/kg	0.025	0.25	1.25	2.5
T_{max}	h	0.04 ± 0.17	0.38 ± 0.35	0.14 ± 0.10	0.27 ± 0.16
C_{max}	ng/ml	484 ± 34	2591 ± 706	3087 ± 699	3983 ± 946
λ_z	h^{-1}	0.09 ± 0.01	0.11 ± 0.04	0.09 ± 0.02	0.10 ± 0.03
$T_{1/2, \lambda_z}$	h	7.6 ± 1.1	7.4 ± 2.9	8.1 ± 1.9	7.3 ± 2.5
AUC_∞	ng · h/ml	3278 ± 283	21409 ± 2068	48790 ± 7539	35749 ± 12501
CL_{tot}	ml/h/kg	7.66 ± 0.65	11.76 ± 1.06	26.01 ± 3.79	75.31 ± 18.46
V_1	ml/kg	52.3 ± 12.1	107.0 ± 14.6	358.7 ± 22.0	522.1 ± 185.9
Vd_{ss}	ml/kg	84.3 ± 12.3	121.4 ± 33.6	506.2 ± 67.3	747.3 ± 106.5
A	ng/ml	245 ± 168	542 ± 429	1398 ± 1345	1599 ± 997
B	ng/ml	249 ± 63	1832 ± 592	2348 ± 112	3557 ± 557
α	h^{-1}	1.47 ± 1.32	0.83 ± 0.72	1.68 ± 0.96	1.41 ± 1.06
β	h^{-1}	0.04 ± 0.04	0.06 ± 0.05	0.06 ± 0.43	0.10 ± 0.03

Pharmacokinetic analysis after intravenous administration was performed using a two-compartment open model described in the text. Each value represents the mean ± S.E. of 4 to 6 rats.

The values of AUC_∞ increased with a rise in the intravenous dose of CoQ_{10}; however, this relationship was nonlinear. The total body clearance, CL_{tot}, increased with a rise in the intravenous dose of CoQ_{10}; as shown in Fig. 2, this relationship was also nonlinear.

In the oral administration study, three different formulations of CoQ_{10} were tested on rats. Figure 3 shows the plasma concentration-time curves of CoQ_{10} after oral administration. The values of AUC_{0-24} and the corresponding absorption rates, Fa, estimated by the Loo-Riegelman[14] method after oral administration of the three different formulations are listed in Table 3. The CoQ_{10} in the control formulation completely dissolved in the olive oil, and CoQ_{10} in the sub-nanosize particle (0.4–8.4 μm; mean particle size, 1.7 ± 0.3 μm) formulation was suspended in water. As shown in Fig. 3, no l-compartment models fitted to the plasma concentration-time data for the orally administered CoQ_{10} formulation. Common among the three formulations was the marked increase in plasma concentration of CoQ_{10} from 10 to 24 hr after administration. The TPGS-emulsion resulted in a much higher AUC_{0-24} value than the other formulations. The values of AUC_{0-24} and Fa were 3.7- and 4.7-fold higher, respectively, than those for the control. There was no difference in the values of AUC_{0-24} and Fa between the control and sub-nanosize particle formulations.

To clarify the absorption site for CoQ_{10} in the intestine, an *in situ* loop study was conducted. The intestinal absorption of CoQ_{10} is shown in Fig. 4. After intraloop administration of the olive oil CoQ_{10} formulation, there were no significant differences in the plasma concentrations of CoQ_{10} among the three formulations (Fig. 4a). The residual amounts of CoQ_{10} in the intestinal loop at the end of the experiment (6 hr after intraloop administration) were almost the same at the three different parts of the intestine (Fig. 4b).

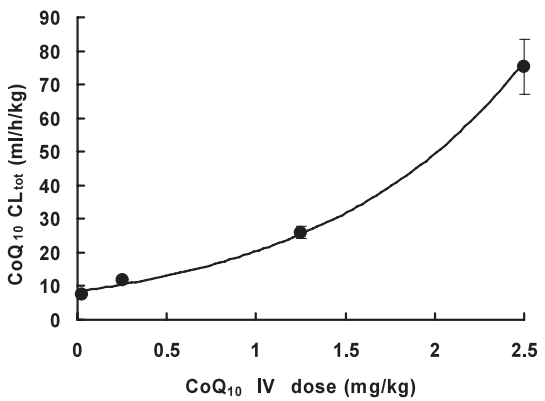

Fig. 2. Total Body Clearance of CoQ_{10} Versus Intravenous CoQ_{10} dose in Rats

The solid line represents a regression line ($Y = 8.409e^{0.886X}$, $R^2 = 0.991$, $p < 0.01$). Each symbol with bars represents the mean ± S.E. of 4 to 6 rats.

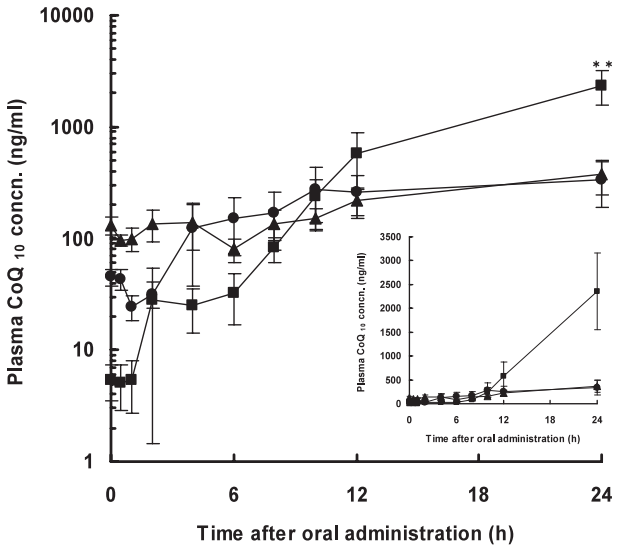

Fig. 3. Plasma CoQ_{10} Concentration Versus Time Curves after Oral Administration of Different Three Formulations

The formulations for oral administration are listed in Table 1. All rats received CoQ_{10} at 75 mg/kg. Key: ●, control; ▲, sub-nanosize particle suspension; ■, TPGS-emulsion. Each symbol with bars represents the mean±S.E. of 6 rats.

Table 3. Comparison of Bioavailability between Formulations Tested in Rats

Formulation[a]		Control	Sub-nanosize particles	TPGS-emulsion
AUC_{0-24}	ng · h/ml	6125 ± 2206	5072 ± 1001	18976 ± 6225**
Fa[b]	%	0.0316	0.0341	0.14942

a) Constituents and how to make them are described in the text. *b*) Fa was calculated by the Loo-Riegelman method using the mean pharmacokinetic data of 0.025 mg/kg intravenous administration. **, $p < 0.01$ against the control.

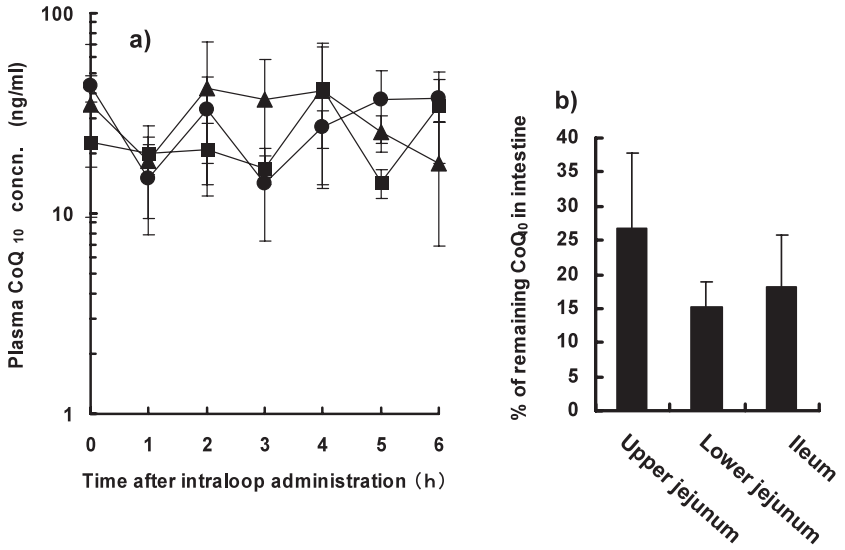

Fig. 4. Plasma CoQ_{10} Versus Time Curves after Intraloop Administration of CoQ_{10} at Three Different Parts of Intestine

At all intestinal parts, 10-cm-long loop was used. Key: ●, upper jejunum; ▲, lower jejunum; ■, ileum. Each symbol with bars represents the mean ± S.E. of 6 rats.

DISCUSSION

CoQ_{10} is an essential cofactor in mitocondrial oxidative phosphorylation, and is necessary for ATP production.[15] CoQ_{10} acts as a mobile electron carrier, transferring electrons from NADH CoQ_{10} reductase or succinate dehydrogenase to the cytochrome b complex.[16] The reduced form of CoQ_{10} is also an antioxidant, and is the only endogenously synthesized lipophilic antioxidant. It can act as an antioxidant directly by protecting biological membranes against oxidants, and can also inhibit the peroxidation of lipoprotein lipids present in the blood.[17] Since CoQ_{10} deficiency in energy metabolism has been shown to be a factor contributing to a number of conditions, many different brands of CoQ_{10} supplement have been used in the treatment of cardiac, neurologic, oncologic, and immunologic disorders, as well as statin myopathy.[18]

However, the bioavailability of CoQ_{10} is extremely low, and CoQ_{10} is absorbed from the intestine at a low rate.[19] The absorption of orally administered CoQ_{10} can be enhanced by interactions with food or food components.[4, 20] In addition, the effect of different formulations of supplements can vary dramatically depending on whether they contain reduced or oxidized CoQ_{10}, whether they are dry powder capsules or CoQ_{10} dispersed in oil, and whether they contain surfactants and emulsifiers, such as lecithin and polysorbate 80, to improve absorption.[21] Moreover, there is also a significant difference in the absorption of CoQ_{10} from orally administered supplements within and between individuals.[22–24]

Although the above points highlight the need for measurement of plasma CoQ_{10} to monitor the efficacy of different modes of preparation and administration, there is little evidence of the pharmacokinetic benefits of CoQ_{10} after intravenous or oral administration. Despite the fact that there are several reports containing the results of pharmacokinetic analyses,[14, 19, 21] no clear consensus has been obtained on whether CoQ_{10} pharmacokinetics can be explained by a certain compartmental model, or on whether the peak plasma concentration of CoQ_{10} can be predicted. From this perspective, in the first part of the study we examined the pharmacokinetic profiles of CoQ_{10} after intravenous administration. As shown in Fig. 1, the plasma CoQ_{10} fitted well with the two-compartment open model, where wellness of curve fitting to the data was judged by a minimum value of Akaike Information criterion.[13] The elimination rate constants or half-life at the terminal phase were almost the same for the four dosing groups, indicating that no saturation process in the metabolism or excretion of CoQ_{10} had occurred. Moreover, CoQ_{10} is metabolized in all tissues in which metabolites are phosphorylated in the cells, transported in the blood to the kidneys, and then excreted into the urine.[25] These observations suggest that CoQ_{10} consumption in tissue cells occurs in a dose-dependent manner without a saturation process, which is another reason to explain the lack of

change in the elimination profiles after intravenous administration of CoQ_{10}. However, a marked dose-dependent increase in the value of CL_{tot} indicates the possibility that a saturation process of protein binding in the blood exists (Fig. 2, Table 2). We initially investigated the protein binding of CoQ_{10} in rat plasma using an ultrafiltration method, but we were unable to detect it because of a marked adsorption of CoQ_{10} at the membrane filter. However, upon a more detailed analysis, we found that the estimated distribution volume in tissues, V_{dss}, at intravenous doses of 0.025, 0.25, 1.25, and 2.5 mg/kg, were 84.3, 121.4, 506.2, and 747.3 ml/kg, respectively. These observations clearly demonstrate that the tissue distribution of CoQ_{10} at higher intravenous doses above 1.25 mg/kg was markedly increased, suggesting the existence of a saturation process in the protein binding of CoQ_{10} in the blood.

Until now, there were no available pharmacokinetic results to characterize the absorption profiles of orally administered CoQ_{10}. It is well known that the profile of CoQ_{10} absorption from the intestinal tract is markedly and widely affected by the presence of food or by biliary excretion of bile acids.[4] Moreover, several clinical trials and case studies have been conducted to support the use of CoQ_{10} in the prevention and treatment of various conditions and disorders related to oxidative stress.[18] However, the large molecular weight (863.63) and lipophilic property of this drug have been shown to limit its oral absorption and consequent efficacy in humans.[26] In those reports, similar values of pharmacokinetic parameters such as C_{max}, T_{max}, and/or absorption rate were not determined because of wide variability in the data collected. In our oral administration study over a 24 hr period, the peak plasma levels of CoQ_{10} and the elimination phase were not detected after oral administration of the three formulations tested (Fig. 3). Mean plasma CoQ_{10} concentrations 10 hr after oral administration ranged from 10 to 100 ng/ml, and this concentration range agreed with the results of other pharmacokinetic studies of CoQ_{10}.[4, 19, 27, 28] However, in the case of all formulations, the plasma CoQ_{10} concentrations 24 hr after oral administration increased again without providing evidence for an elimination phase. Since the absorption process of CoQ_{10} from the intestinal tract is carried out by micelles,[4] the formation of micelles and the incorporation of poorly water-soluble drugs into micelles are considered to be important factors affecting the absorption.[29] Therefore, regarding the effect of food intake on the plasma concentration of CoQ_{10} after oral administration, it is possible that some food components or bile acids play an important role in the intestinal absorption of CoQ_{10}.

In this study, however, we investigated the absorption profiles of CoQ_{10} from the intestinal tract after oral administration in a fasted condition. Therefore, the effects of micelle formation or food-intake on the absorption profiles were negligible. As shown in Fig. 3, although there were no differences in plasma CoQ_{10} concentrations within 10 hr of oral administration of the three formulations, we found that the plasma concentration from the TPGS-emulsion at 24 hr after administration showed approximately a 7-fold increase, and the AUC_{0-24} of this formulation was 3.7-fold higher than that of the control. The Fa of the TPGS-emulsion, which was estimated using a deconvolution method, was 4.7-fold higher than that of the control (Table 3). These two values are of the same order of magnitude, and suggest that the intestinal absorption of CoQ_{10} from the TPGS-emulsion improves the CoQ_{10} plasma concentration more than the intestinal absorption of CoQ_{10} after oral administration. Wajda *et al.* reported that CoQ_{10} and vitamin E formulated in Nanosolve® (Lipoid GmbH, Ludwigshafen, Germany) improved the bioavailability of CoQ_{10} after oral administration 5-fold.[28] In this formulation, CoQ_{10} and vitamin E were emulsified by purified phospholipids obtained by extraction from soybeans, where the phospholipids also act as an emulsifier. TPGS is a water-soluble form of vitamin E modified by polyethylene glycol 1000 succinate.[30] TPGS acts as an absorption enhancer to improve the intestinal absorption of cyclosporine, either by decreasing transport back into the intestine by P-glycoprotein (P-gp) or by affecting some unknown mechanism by which cyclosporine is protected from metabolism in the gut.[29] In addition, CoQ_{10} interaction affects the transport activity of P-gp, and the efflux transport of CoQ_{10} is mediated by P-gp in Caco-2 cells.[31] From these observations, it can be concluded that use of an emulsion formulation with TPGS as an absorption enhancer improves the bioavailability of CoQ_{10} after oral administration more than use of a sub-nanosize particle suspension formulation or an oil-mixed formulation.

To confirm the factors variation in the oral absorption profiles of CoQ_{10}, we tried to determine the intestinal absorption site of CoQ_{10} using an *in situ* loop study. As shown in Fig. 4, there were no

significant changes in the plasma CoQ_{10} levels after intraloop administration of CoQ_{10} in olive oil. Moreover, the residual amounts of CoQ_{10} in different parts of the loop (upper and lower jejunum, and ileum) 6 hr after administration were almost the same. These findings indicate that there is no specific absorption site for CoQ_{10} in the intestine. Taking the results from Figs. 1, 2, 3, and 4 into consideration, we speculate that there is a deep compartment for CoQ_{10} accumulation in the intestine such as intestinal membranes and lymphatic vessels.

In summary, we have undertaken pharmacokinetic and pharmaceutical study of CoQ_{10}. CoQ_{10} shows nonlinear kinetics, which may be caused by the saturation of protein binding in the blood. In addition, the CoQ_{10} absorption process takes a long time, and we speculate that this may be due to a deep compartment for CoQ_{10} accumulation in the intestine. Absorption of CoQ_{10} from the intestinal tract was very poor; however, a higher plasma concentration of CoQ_{10} was achieved by an emulsion formulation using TPGS.

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